PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 241/08, 295/02, A61K 31/495	A1	(11) International Publication Number: WO 97/10222 (43) International Publication Date: 20 March 1997 (20.03.97)

(21) International Application Number: PCT/US96/14569

(22) International Filing Date: 11 September 1996 (11.09.96)

(30) Priority Data:
08/527,407 13 September 1995 (13.09.95) US

(71) Applicant: CORTECH, INC. [US/US]; 6850 North Broadway, Denver, CO 80221 (US).

(72) Inventors: GOODFELLOW, Val, S.; 10045 S. Hooker Court, Westminster, CO 80030 (US). MCLEOD, Donald, A.; 10439 Iris Way, Westminster, CO 80021 (US). GERRITY, James, Ivan; 13275 W. 65th Drive, Arvada, CO 80004 (US). LAUDEMAN, Christopher, P.; 4622 S. Pagosa Circle, Aurora, CO 80015 (US). BURKARD, Michael, R.; 3866 W. 126th Avenue, Broomfield, CO 80020 (US).

(74) Agent: BIRD, Donald, J.; Pillsbury, Madison & Sutro, Cushman, Darby & Cushman Intellectual Property Group, 9th floor, 1100 New York Avenue N.W., Washington, DC 20005-3918 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR PREPARING PIPERAZINES

(57) Abstract

The invention provides a method of synthesizing N-substituted 1,4-piperazines and N-substituted 1,4-piperazinediones. The invention also provides a method for synthesizing combinatorial libraries of piperazine derivatives which can be assayed for biological activity in prespecified ligand binding or enzymatic activity screens.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

METHOD FOR PREPARING PIPERAZINES

Field of the Invention

This invention relates to a method of generating large numbers of diverse non-peptide organic compounds which can be assayed for biological activity in pre-specified ligand binding or enzymatic activity screens. More specifically the diverse organic compounds formed are N-substituted 1,4-piperazines or their corresponding piperazinediones formed by "on-resin" or solid-phase cyclization of unnatural N-substituted dipeptoids. These compounds are useful in that they serve as an inexpensive source of chemical diversity to identify low molecular weight lead compounds with desired ligand affinity or enzyme inhibitory activity, which can be further optimized by combinatorial or traditional medicinal chemical methodology.

Background of the Invention

Traditionally the most effective strategies for identifying novel non-peptide lead structures which have affinity for a targeted receptor or enzyme, which normally binds peptidic ligands, have included mass screening of diverse compounds. Mixtures of natural products, usually obtained as extracts, or chemical files of synthetic compounds are traditionally the source of diverse chemical structures for lead identification. Often the lead compound may possess relative weak affinity for the targeted receptor or enzyme; the affinity can then be increased by systematic, empirical modification of the lead structure.

Lead compounds are found by screening of natural products obtained from plant, soil, or animal sources, or fermentation with micro-organisms in diverse media. Synthetic compounds contained in historical collections of pharmaceutically interesting molecules or their synthetic precursors are also screened. Both natural and synthetic sources of chemical diversity present their own unique problems and limitations. Unfortunately natural product leads are often extremely difficult to identify, purify, and

synthesize. Increasingly the use of natural products leads creates ethical dilemmas in that the organism which produces the lead compound may be rare and the discovery of a new source of biological activity may endanger the natural specie which produces the chemical, or the biome where the specie is found. Historical chemical libraries collections produced by pharmaceutical companies are often limited in diversity and scope since the original compounds were originally designed to target specific diseases or pathophysiological mediators, and more importantly diverse collections chemical files are unavailable to researchers who do not possess enormous financial resources.

Synthetic combinatorial libraries of diverse peptides have only partially answered the need for new methods to identify novel ligands for pharmaceutically interesting targets. Peptides suffer from a number of disadvantages including poor oral bioavailability, poor in vivo stability and high cost. Although a very large number of diverse peptides can be made, their structural diversity is limited to natural or readily synthesible amino acid building blocks and their linear polymeric character. The transformation of a peptide lead into an orally available, biologically stable, clinically useful drug, is an extremely time consuming and expensive research project and has met with limited success.

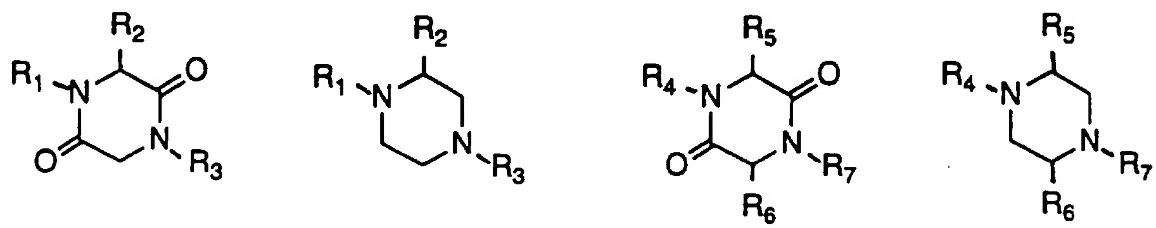
Recently several solid phase combinatorial approaches have been developed for creating diverse libraries based on small scaffolds which have been previously incorporated in successful drugs. These scaffolds include heterocycles such as benzodiazepines (Bunin et al., J. Am. Chem. Soc. 114 10997 (1992)), hydantoins (DeWitt, Proc. Natl. Acad. Sci. USA 90 6909 (1993)) and piperazinediones (Gordon et al., Biorganic and Medicinal Chemistry Letters, 5 47 (1995)) and non-heterocyclic scaffolds such as biphenyls (Pavia et al, PCT/US94/07780 (WO 95/04277). The present invention provides a method for producing 1,4-piperazine-2,5-diones or their corresponding reduced 1,4-piperazine analogs with greater potential for diversity in structure and with a chemical synthesis which offers advantages in yield, purity, cost and ease of production over known methods of producing diverse piperazinediones.

Piperazines and piperazinediones are common scaffolding structures found in a wide variety of bio-active compounds and drugs. Examples of this disclosed invention have been found to bind to human

bradykinin , human neurokinin 1 and 2, human mu and kappa (animal) opioid receptors, and are expected to function as antagonists in many G-protein coupled receptors or inhibitors of many classes of enzymes.

Detailed description of the invention

The present invention comprises a method for producing large numbers of structurally diverse piperazines and piperazindiones of the general structures illustrated below.



Where R^1 through R^7 are independently selected from hydrogen or C1 to C12 alkyl, C3 to C8 cycloalkyl, or alkylaryl pendant groups. The pendant groups may be substituted with guanidino, alkylguanidino, benzamidino, methoxy, ethoxy, methyl, ethyl, propyl, butyl, piperidinyl, piperazinyl, indanyl, indolyl, quinuclidinyl, hydroxyl, amino, alkylamino, aminoalkyl, biphenyl, napthyl, imidazoyl, pyridinyl, purinyl, benzoxazolyl, carboxamides, sulfonamides, carboxylates, pyrazoles, pyrimidines, fluoro, chloro, bromo, or trifluromethyl moeities.

In a preferred embodiment of the invention R^1 is selected from cyclohexyl, methylcyclohexyl, benzyl, substituted benzyl, phenethyl, biphenyl, benzoyl, hydrogen, methylnapthyl, indanyl, guanidinoalkyl (C2 to C8), aminoalkyl (C2 to C8), arylalkylcarbamates with alkyl chains of C2 to C8, alkanoyl (C1 to C12), and alkyl (C1 to C12) or hydrogen.

In a preferred embodiment of the invention R^2 is selected from benzyl, substituted benzyl, indole, methylnapthyl, cyclohexyl, methylcyclohexyl, indanyl, (C1 to C5)alkyloxyaryl, aminoalkyl (C1 to C8), guanidino alkyl (C1 to C8), alkylcarboxylic,hydroxyalkyl(C1 to C5), alkylthioalkyl, (C1 to C3) alkylbenzamidino, or hydrogen.

In a preferred embodiment of the invention R^3 is selected from is selected from cyclohexyl, methylcyclohexyl, benzyl, substituted benzyl,

phenethyl, biphenyl, benzoyl, hydrogen, methylnapthyl, indanyl, guanidinoalkyl (C2 to C8), aminoalkyl (C2 to C8), arylalkylcarbamates with alkyl chains of C2 to C8, alkanoyl (C1 to C12), alkyl (C1 to C12), (C2 to C12)-alkylguanidinoalkyl(-C2 to C8), benzamidino, (C1 to C6) alkylbenzamidino, (C1 to C6)alkylquinuclidine, (C1 to C6) alkyl piperazine, C1 to C6 alkyl N-substituted piperazine, (C1 to C6) alkylamino piperidine, (C1 to C6) alkylamino N-substituted piperidine (C1 to C6) alkylcarboxyamidobenzamidino, (C1 to C6)alkylcarboxamidoquinuclidine, (C1 to C6) alkylcarboxamido piperazine, C1 to C6 alkylcarboxyl N-substituted piperazine, (C1 to C6) alkylcarboxamidino piperidine, or (C1 to C6) alkylcarboxamidino N-substituted piperidine, or hydrogen.

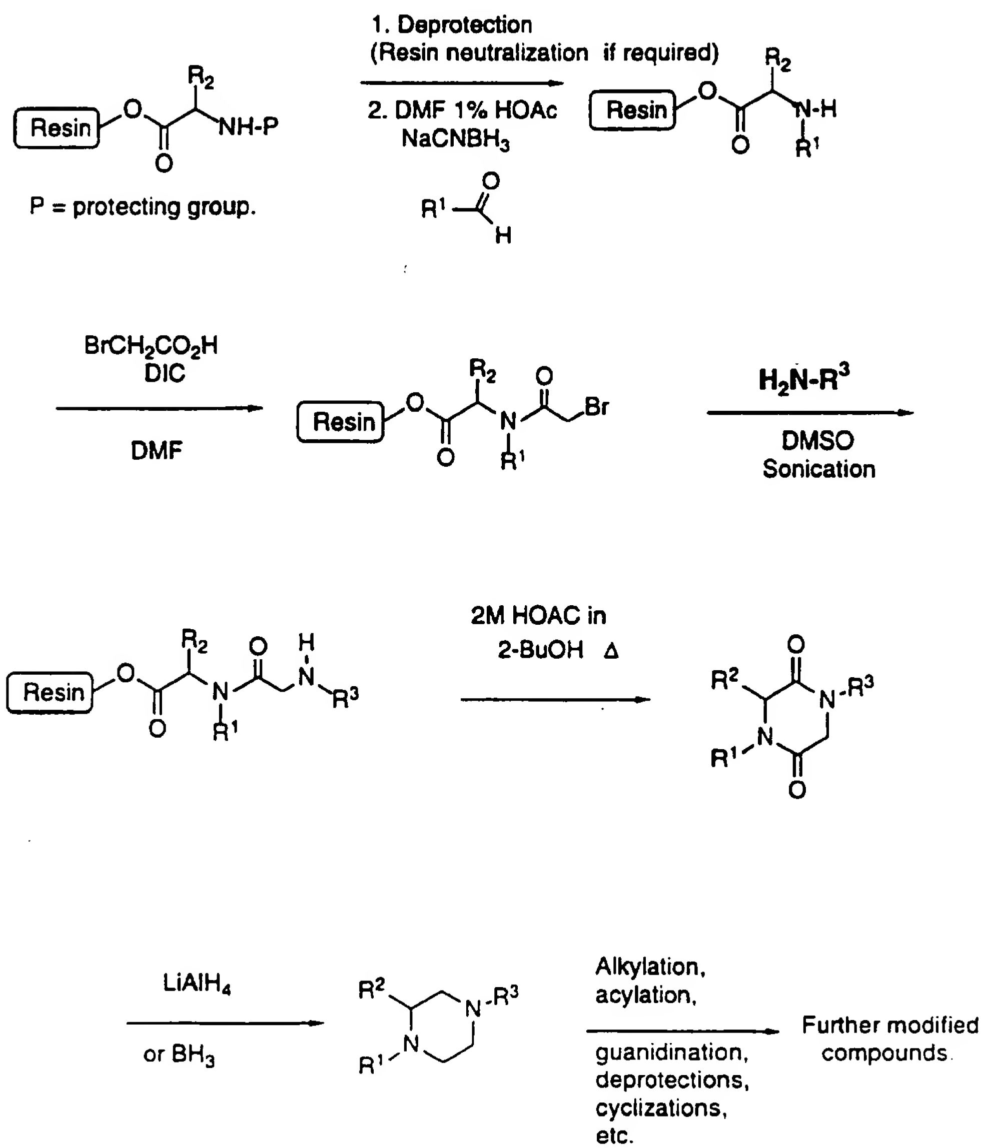
In another preferred embodiment R^4 and R^7 are independently defined as for R1; and R^5 and R^6 are independently defined as for R^2 or R^3. In this disclosure two most preferred methods for synthesizing independent purified piperazinediones and piperazines are described. A preferred method for preparing a diverse combinatorial library of piperazinediones and piperazines with several thousand discrete components from which lead bradykinin antagonist structures and other bio-active compounds with G-protein binding properties have been discovered is also disclosed.

The compounds or libraries of compounds of this invention are preferably synthesized as described in schemes I and II below. The compounds of the invention could also be synthesized by those skilled in the art by adaptation of the solid phase methodology described here to solution phase methods; such an adaptation would be considered an extension of the current invention.

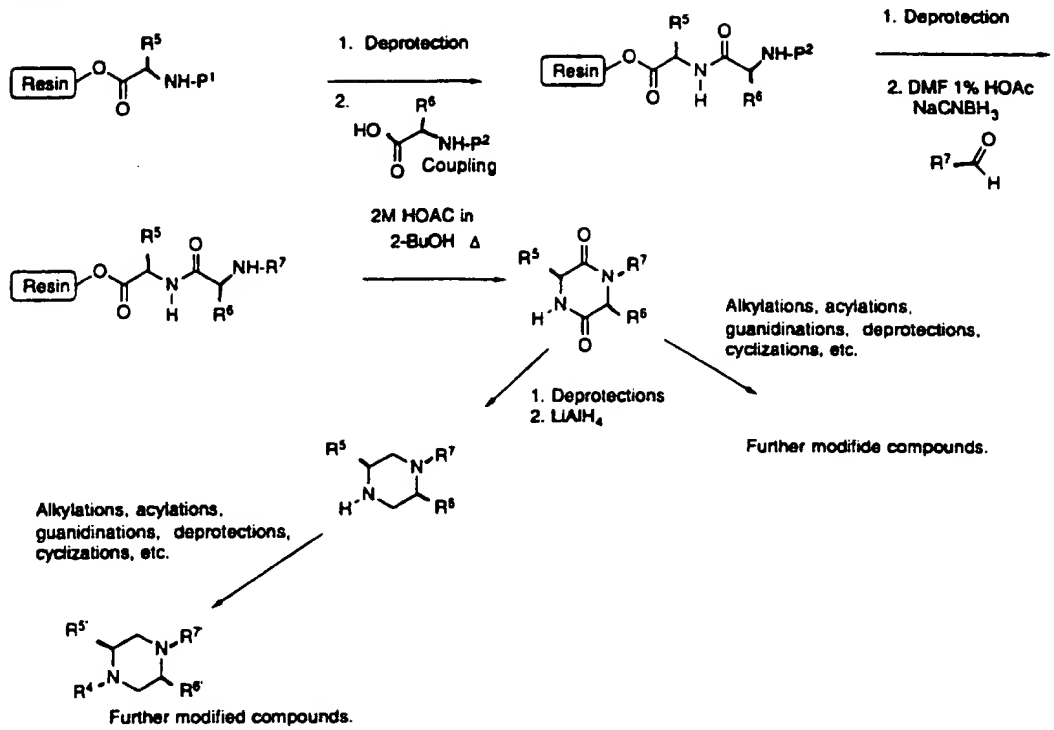
A suitably N-protected α-amino acid is attached to a polymeric support using methods well known in the art. These methods include displacement of benzylic halogens to form ester linkages utilizing cesium carbonate, potassium carbonate or potassium iodide to accelerate the reaction. Ester formation can also be accomplished by the action of carbodiimide coupling reagents such as dicylohexylcarbodiimide or diisopropylcarbodiimide and a benzylic alcohol, utilizing tertiary base, with or without 4-dimethylaminopyridine as catalyst. Reagents such as BOP, HBTU, and BOP-Cl can also effectively be used to derivatize the resin. An advantage of the current invention is that the resin need not be a

benzylic ester support such as Merrifield or Wang type resins, since acidolysis is not required to free the piperazinedione from the resin. The solid support may be an alkyl derivative such as polyethylene glycol or any other polymeric or discrete hydroxyl group. A very large number of suitably N-protected resin-immobilized α-amino acids are commercially available and all but the most acid sensitive of these constructs are applicable in the current invention.

Scheme I



Scheme II



In method A, illustrated in scheme I, the resin immobilized amino acid is deprotected according to methods well known in the art. These methods may include treatment with trifluoroacetic acid for Boc-protected amino acids, treatment with secondary amines such as piperidine for Fmoc-protected amino acids or treatment with hydrogen or organic-palladium complexes for reducible protecting groups. The resulting free amino group is then reductively alkylated with a suitable adehyde, preferably by treatment with $NaCNBH_3$ in the presence of catalytic acetic acid in dimethylformamide. A number of alternative methods for accomplishing reductive amination are known in the art and include hydrogenation in the presence of soluble organic substituted metal catalysts, variously substituted borohydride reagents or the use of Lewis Acids such as $Ti(O\text{-}Pr)_4$ and sodium cyanoborohydride. The resulting secondary amine is facilely bromoacetylated with bromoacetic acid and diisopropylcarbodiimide or dicyclohexylcarbodiimide. This reaction can also be accomplished with other coupling reagents such as HBTU. The resulting bromoacetyl compound is then reacted with concentrated primary amines in a polar aprotic solvent such as DMSO to provide an N-substituted

glycyl residue. The key step to the synthesis in this disclosure is the solid-phase mediated simultaneous cyclization and cleavage of the incipient dipeptoid from the solid phase resin. This is accomplished by heating in a non-reactive solvent such as 2-butanol in the presence of a catalyst such as acetic acid. The boiling point, polarity, solvent characteristics and hydroxylic character of racemic 2-butanol all contribute to the success of the cyclization method. The simultaneous cyclization and release of piperazinediones has numerous advantages:

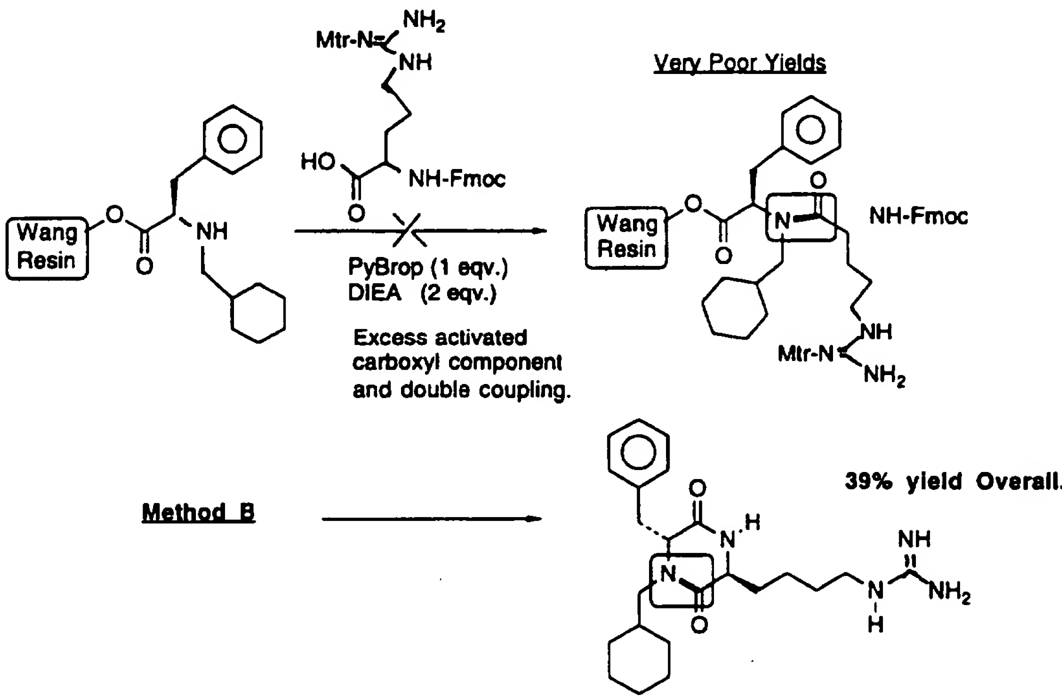
1. Since compounds which are not dipeptoids are not released from the resin by these mild conditions, failure sequences or potential impurities are not freed from the resin to contaminate the desired piperazinediones. Crude purities, following aqueous work-up, in excess of 95% can be obtained in selected cases.

2. The mild deprotection chemistry involved allows a very large number of acid sensitive substituents and acid sensitive protecting groups to be employed, we have routinely produced diketopiperizines containing versatile protecting groups such as the Boc and Cbz group which were then removed for further modification of the molecule.

3. The reaction scheme is simple and provides compounds of superior purity without resorting to expensive and sensitive reagents such as PyBrop or its relatives or corrosive acids such as TFA; such considerations increase the propensity for the application of this method to automated reaction devices.

4. Since the cyclization/cleavage reaction is driven by high temperature and is a highly favored intramolecular formation of a piperazindione, amide bonds can be formed which are very difficult to form by alternative methods. For example the solution and solid phase formation of the dipeptoid illustrated was attempted using a number of reagents including PyBrop, the reagent of choice for the synthesis of peptoids involving couplings at hindered secondary amines and suggested for the solid-phase synthesis of

piperazinedione libraries, (D. W. Gordon Biorganic and Medicinal Chemistry Letters, 5 47 (1995)) in our hands we obtained essentially none of the desired compound. Utilizing the methodology of this invention (method B) a corresponding difficult amide bond was formed very readily during the cleavage step. Consequently we believe that piperazindiones are readily available utilizing this methodology which can not be easily formed by other reported methods which involve couplings of very hindered secondary amines.



An additional advantage is accrued by the addition of the R^3 substituent by displacement with a primary amines via method A. The introduction of complex groups via primary amines greatly increases the diversity of substituents for the side chain over methods which only employ aldehydes as in reductive alkylation reactions. The supply of commercial primary amines is much more diverse than less stable commercial aldehydes. A very large number of suitable primary amines are available commercially or through simple synthetic steps making an enormous number of diverse compounds possible. The geometric orientation of such

N-substituted species offers increased diversity over piperazinediones obtained from simple dipeptides.

In method B a dipeptide may be synthesized on a supporting resin by methods well known in the art, deprotection of the dipeptide produces an N-terminal amine which is reductively alkylated as described in method A. Again the key step to the synthesis in this invention is the solid-phase mediated simultaneous cyclization and cleavage of the incipient dipeptoid from the solid phase resin. This is accomplished by heating at reflux in a non-reactive solvent such as 2-butanol in the presence of a catalyst such as acetic acid. As in method A a large number of additional reactions and deprotections can subsequently be carried out on the free diketopiperazines. We illustrate a number of deprotection reactions, acylations and guanidination reactions.

Piperazinediones produced by either method A or method B can be reduced to the corresponding piperazine structure by treatment with strong reducing agents such as diborane or lithium aluminum hydride.

In many instances the compounds that are produced are of high purity and may be assayed directly or they may be purified by common chromatographic techniques such as preparative reverse phase high performance chromatography or ion exchange chromatography.

In order to produce large numbers of diverse analogs, mixtures of precursor reagents may be formed which produce mixtures of compounds which differ in structure in the various side chain substitutions. A simple method for producing mixtures of controlled content is to derivatize individual resin samples with a given amino acid substituent and then mix portions of such resins, each portion derivitized with a unique amino acid substituent. The resins are then split into aliquots and reacted with different reagents which produce a variety of substituents at one given position. Each aliquot is then split into new aliquots which are reacted with reagents which provide a variety of substituents at a new position. In the examples of this patent we illustrate this process by providing a resin mixture which is derivatized by 10 different amino acid derivatives. (This number could be much larger, but a simple example is chosen for clarity.) This resin is split into 12 aliquots. Each of 10 aliquots is reductively alkylated with a unique aldehyde. Each resulting aldehyde is split after bromoacetylation into 12 new aliquots, each of which is reacted with a

unique primary amine. The resulting compound is then cyclized and each resulting mixture is split in half, half of the aliquot is then reduced with lithium aluminum hydride to provide piperazines. Two of the original aliquots are not reacted with aldehydes but are bromoacetylated, reacted with amines, cyclized, reduced to piperazines first and then acylated with either benzoic acid or dodecanoic acid. The various processes illustrated here produce a large number of mixtures of compounds, where each mixture contains at least 10 unique piperazines or piperazindiones. These mixtures are conveniently assayed by HPLC and MALDI -Mass Spectrometry, and then tested after dissolution in DMSO for activity in selected biological assays. We illustrate broad screening on a human bradykinin receptor clones. Human neurokinin type I and II receptor clones, human mu and guinea pig brain kappa opioid receptor clones. Compounds can be selected for specific identification and resynthesis on the basis of an apparent level of inhibition at a given concentration.

This process is concisely illustrated by the discovery of two bradykinin human receptor ligands as potentially pharmaceutically useful leads for the development of potent bradykinin antagonists. Screening of example combinatorial mixture 219 at an average estimated concentration of 10 uMolar produced 77% inhibition of radio-labeled bradykinin binding in the human receptor clone assay. This sample was subjected to a 5-60% CH3CN, 30 minute, then 60-90%, 15 minute gradient in water containing constant 0.1% TFA on a 25 mm reverse phase HPLC column, (10 mL/min flow rate). Fractions of approximately 10 mL were collected. Aliquots of the collected fractions were rescreened for BK binding inhibition and fraction 34 exhibited 65% inhibition. The active component of fraction 34 was identified with MALDI Mass spectrometry as example 60. This compound was resynthesized in pure form according to method A, and assayed for BK binding inhibition, an IC_{50} value of 4.1 uMolar (human BK_2 receptor) was obtained. In an analogous fashion, combinatorial mixture example 166, exhibited 71% inhibition at an estimated average concentration of 10 uMolar. The sample was submitted to chromatography, binding assays of fractions, mass-spectrometry and the example 61 was identified as the active component and synthesized in pure form by method A. An IC_{50} value of 13 uMolar (human BK_2 receptor) was obtained.

Numerous other human receptor ligands have been identified from this single combinatorial library set.

Examples

Example 1. General Procedure for Resin Derivitization:

In a solid phase peptide synthesis device (Stewart et al., Solid Phase Peptide Synthesis, Pierce Chemical Company, 1984), pre-silanized with Sigmacote, Wang peptide synthesis resin (p-benzyloxybenzyl alcohol [copolystyrene-1%-divinyl benzene] resin, 0.2 to 0.80 meqv. / g), 300 mg to 10 g is suspendend in DMF (10 volumes) and then the solvent is filtered away. The resin is washed two additional times with DMF. The resin is washed three times with dichloromethane, then three times with DMF. Finally the resin is suspended in DMF and allowed to mix with nitrogen bubbling for 30 to 60 minutes. In a separate flask 5 to 10 equivalents of N$^{\alpha}$-Fmoc-Protected amino acid is dissolved in the minimum volume of dry DMF and treated with 0.2 equivalents 4-dimethylaminopyridine, and 10 equivalents of N,N'-diisopropylcarbodiimide. The reaction is transferred to the synthesis reaction vessel containing the Fmoc-protected Amino Acid resin and the mixture is agitated for 15 hours with nitrogen bubbling. The reaction mixture is filtered away and the resin is washed sequentially, with DMF, methanol, DMF, and dichloromethane, the resin is dried in a stream of dry nitrogen, then under high-vacuum. The amount of Fmoc-amino acid added to the resin is estimated colorimetrically as follows: A 5 mg sample of the resin was suspended in 6.0 mL of DMF containing 20% piperidine. After 10 minutes of gentle agitation, the solution is decanted and the absorbance measured at 290 nM. The measured absorbance at 290 nM is multiplied by 0.2424 to yield the resin substitution in units of miliequivalents per gram of derivatized resin.

Example 2. General Procedure for Synthesis of Piperazinediones According to Method B.

A. Resin Deprotection

The resin immobilized Nα-Fmoc-amino acid (side-chain R^5) is deprotected by reaction with 25% piperidine in DMF (~ 20 mL / g resin)

with agitation by nitrogen bubbling for 25 minutes. The solution is filtered away and the resin is reacted again with 25% piperidine in DMF (~ 20 mL / g resin) with agitation by nitrogen bubbling for 25 minutes. The solution is filtered away and the resin is washed well with DMF.

B. Amino Acid Activation

Symmetrical Anhydride Method: In a separate flask 4 equivalents of the Fmoc-amino acid (side-chain R^6) are dissolved with warming in the minimum volume of dichoromethane containing 10% DMF, the solution is cooled to room temperature and 2 equivalents of dicyclohexylcarbodiimide are added. The reaction is stirred for 30 minutes at room temperature; the resultant dicyclohexylurea (DCU) is filtered away and the filtrate is added to the resin prepared above. The reaction mixture is agitated for 2 hours with nitrogen bubbling.

Benzotriazole Active Ester Method:

Difficult couplings such as those employing Fmoc-Igl-OH can be accomplished by preforming the active ester by the reaction of four equivalents of the Fmoc-amino acid in DMF with four equivalents of hydroxybenzotriazole and dicyclohexyl carbodiimide. After 30 minutes (DCU) is filtered away and the reaction mixture is added to the resin as above. Similar preactivation with HBTU/HOBt and 8 equivalents diisopropylethyl-amine is acceptable for very difficult couplings.

C. Resin Deprotection:

As described in step A.

D. Reductive Amination

Dried resin is suspended in DMF and agitated for 30 minutes; the DMF is filtered away and the resin is then treated with DMF (~ 40 mL /mmole) containing 1% acetic acid. Three equivalents of the appropriate aldehyde is added followed by 10 equivalents of sodium cyanoborohydride. The reaction is agitated by nitrogen bubbling for 6 to 24 hours. Reaction progress can be accessed by the Kaiser test (Ibid pg 76) or by HPLC analysis of a TFA cleavage reaction of a few resin beads. If incomplete reaction is obtained the reaction is filtered and resubmitted to the reductive amination

conditions. When complete reductive amination is achieved the reaction mixture is filtered and washed well with DMF then dichloromethane, followed by another series of DMF washes. The resin may be dried if storage is required after the dichloromethane washes.

E. Cyclization:

Dried resin or slurried aliquots are transferred to a round bottom flask or mini-reactor tube and treated with 2-butanol containing 11% (v/v) acetic acid (~ 5 to 10 mL /mg resin). The reaction vessel is placed in a 110 ° oil bath and heated at reflux under nitrogen for 48 to 72 hours. The crude piperazinedione is isolated by filtration, and washing of the resin with acetonitrile, methanol, and dichloromethane. The volatile solvents are removed by rotary evaporation. The product may be purified by HPLC chromatography. Side chain protecting groups such as Cbz and Boc are conveniently deprotected at this stage, before $LiAlH_4$ reduction. Free amino groups formed by deprotection may be acylated or guanidinated.

Example 3. General Procedure for Guanidination of Side-Chain Amino Groups Derived from N-Boc-Protected Moieties.

The crude diketopiperazine obtained by removal of the 2-butanol/acetic acid solution is dissolved with excess (>15 equivalents) of trifluroacetic acid as a 50% v/v solution in dichloromethane. The reaction is stirred one hour to insure removal of the side chain N-Boc protecting group. The solvent is then removed on the roto-vap. The residue is treated with dichloromethane and the solvent is again removed using rotary evaporation. The sample is again treated with dichloromethane and the solvent removed using rotary evaporation. The residues are dissolved in DMF (0.1 to 1 mL/ 20 mg crude product) and treated with two equivalents of pyrazole-1-carboxamidine hydrochloride (Bernatowicz et al., J. Org. Chem. 57 2497 (1992)) and 5 equivalents of N,N-diisopropyl-ethylamine. The reaction is stirred 6 to 24 hours and then is treated with excess TFA in dichloromethane. The solvent is evaporated and the crude product is precipitated with anhydrous diethylether and purified by reverse phase HPLC chromatography. Primary amino groups in piperazines produced by

reduction of piperazinediones by $LiAlH_4$ are guanidinated using the identical procedure in DMF with two equivalents of pyrazole-1-carboxamidin hydrochloride and 5 equivalents of N,N-diisopropyl-ethylamine. The reaction is stirred 6 to 24 hours and then treated with excess TFA in dichloromethane. The solvent is evaporated and the crude product is precipitated with anhydrous diethylether and purified by reverse phase HPLC chromatography.

Example 4. General Procedure for Guanidination of Side-Chain Amino Groups Derived from N-Cbz-Protected Moieties.

The crude diketopiperazine obtained by removal of the 2-butanol/acetic acid solution is suspended in acetic acid (~0.5 to 1 mL / 100 mg crude product) and treated with excess (>15 equivalents) of hydrobromic acid in a 30% solution in acetic acid. The reaction is stirred for 30 minutes to insure removal of the side chain N-Cbz protecting group. The crude product is precipitated by addition of anhydrous diethylether and collected by centrifugation. The primary amine (as the HBr salt) is then guanidinated as described in example 4. In general compounds are purified by reverse phase HPLC chromatography although crude puritys of 95% have been obtained.

Example 5. General Procedure for $LiAlH_4$ Reduction of Piperazinediones from Method A to Piperazines.

The crude piperazinedione after cyclization or side-chain deprotection is dissolved under nitrogen with 20 equivalents of a 1 M $LiAlH_4$ solution; the solution is then heated at reflux under nitrogen for 24 hours. The reaction is quenched with ice and the volatiles removed by rotary evaporation. The residue is extracted with acetonitrile, filtered, acidified with TFA to ~pH 3, and the product purified by reverse phase HPLC or Sep-Pak filtration using a step gradient of 0-50% acetonitrile.

Example 6. General Procedure for $LiAlH_4$ Reduction of Piperazinediones from Meth d B t Piperazines.

The crude piperazinedione after cyclization or side-chain deprotection is dissolved under nitrogen with 20 equivalents of a 1 M $LiAlH_4$ solution; the solution is then heated at reflux under nitrogen for 24 hours. The reaction is quenched with ice and the volatiles removed by rotary evaporation. The residue is extracted with acetonitrile, filtered, acidified with TFA to ~pH 3, and the product purified by reverse phase HPLC or Sep-Pak filtration using a step gradient of 0-50% acetonitrile.

Example 7. General Procedure for Acylation of Piperazines.

Piperazines containing primary or secondary amines are dissolved in DMF (0.05 to .5 molar) and treated with 1.25 equivalents of the appropiate carboxylic acid, 1.25 equivalents of diisopropyl-ethylamine and 1.25 equivalents of HBTU. The reactions are agitated, stirred or sonicated for 2 to 16 hours in polypropylene conical capped tubes. Each tube is treated with a mixture of 1 N NaOH and EtOAc. After vigorous mixing, the aqueous layer is removed by suction through a capillary, and the NaOH wash repeated. The organic layer is evaporated utilizing a speed-vac (high speed centrifugal vacuum evaporator) and the samples are purified by HPLC or step- wise gradient (0 - 80% acetonitrile in water) on a Sep-Pak column. Samples are evaporated utilizing the speed-vac, and dissolved in DMSO for biological testing.

Example 9. General Method for Synthesis of Piperzinones According to Method A.

The N^{α}-Fmoc- amino acid derivitized resin is treated with DMF in a silanized (Sigmacote) reaction vessel, and agitated for 30 minutes. The DMF is filtered away and the resin is treated with 25 % piperidine in DMF (~20 mL / g resin). The deprotection reaction is agitated with nitrogen for 25 minutes. The solvent is then filtered away, and the resin reacted again for 25 minutes with 25% piperidine in DMF with nitrogen bubbling. The reaction mixture is filtered away and the resin is washed well with DMF, dichloromethane, methanol, and dichloromethane. The resin can then be stored under nitrogen at low temperature. Dried resin is suspended in DMF and agitated for 30 minutes; the DMF is filtered away and the resin is

then treated with DMF (~ 40 mL /mmole) containing 1% acetic acid. Three equivalents of the appropriate aldehyde is added followed by 10 equivalents of sodium cyanoborohydride. The reaction is agitated by nitrogen bubbling for 6 to 24 hours. Reaction progress can be assessed by the Kaiser test or by HPLC analysis of a TFA cleavage reaction of a few resin beads. If incomplete reaction is obtained, the reaction is filtered and resubmitted to the reductive amination conditions. When complete reductive amination is achieved the reaction mixture is filtered and washed well with DMF then dichloromethane, followed by another series of DMF washes. The resin immobilized N-substituted amino acid is then bromoacetylated with a solution of 12 equivalents of bromoacetic acid and 13.2 equivalents of diisopropyl-carbodiimide in DMF (~ 10mL /g resin). The reaction is agitated for 30 minutes, filtered, washed with DMF, and treated a second time with 12 equivalents of bromoacetic acid and 13.2 equivalents of diisopropyl-carbodiimide in DMF (~ 10mL /g resin). After 30 minutes of nitrogen agitation the solution is filtered, washed well with DMF, dichloromethane and dried under a nitrogen stream. The resin is then dried under vacuum. The resin is treated with a solution of an appropriate primary amine (0.25 to 2 M) in DMSO (~ 1 mL / 100 mg of resin). The reaction may be sonicated for 3 hours or agitated for approximately 15 hours. The mixture is then filtered, washed well with DMF, dichloromethane, methanol, and finally with dichloromethane. The derivatized resin is then dried in a stream of nitrogen and then under vacuum if storage is necessary. The dried resin or slurried aliquots are transferred to a round bottom flask or mini-reactor tube and treated with 2-butanol containing 11% (v/v) acetic acid (~ 5 to 10 mL / mg resin). The reaction vessel is placed in a 110 ° oil bath and heated at reflux under nitrogen for 48 to 72 hours. The crude piperazinedione is isolated by filtration, and washing of the resin with acetonitrile, methanol, and dichloromethane. The volatile solvents are removed by rotary evaporation. The product may be purified by HPLC chromatography. Side chain protecting groups such as Cbz and Boc are conviently deprotected at this stage, before $LiAlH_4$ reduction. Free amino groups formed by deprotection may be acylated or guanidated.

Example 10. General Method for Combinat rial Synthesis of Mixtures of Piperzin nes According to Method A.

A large combinatorial library of piperazines and piperazinediones was synthesized as follows.

A. Resin Mixing, Deprotection and Slurry Splitting:

The following N^{α}- Fmoc amino acid resins were mixed in a large silanized solid phase peptide synthesis apparatus.

Fmoc-Amino Acid	Loading (meq/g)	mMoles	Amount used.
L-Phenylalanine	0.72	0.625	0.87 g
D-Phenylalanine	0.81	0.625	0.77 g
L-Tryptophan	0.66	0.625	0.95 g
D-Tryptophan	0.57	0.625	1.10 g
D,L- Cha	0.57	1.25	2.20 g
D,L - 2-Nal	0.55	1.25	2.28 g
D,L- Igl	0.50	1.25	2.50 g
10 (5 D,L Pairs) Total	0.625 Average	6.25 Total	10.67 g total

The resins were suspended in DMF and agitated for 30 minutes, the DMF was filtered away and treated with 200 mL of 25% piperidine in DMF with nitrogen agitation for 25 minutes. The solution was drained away and the resin was again treated with 200 mL of 25% piperidine in DMF with nitrogen agitation for 25 minutes. The solution was filtered away and the resin was washed well with DMF and dichloromethane. The resin was slurried in a mixture of 3:2 dichloroethane:dimethylformamide (V/V), and the the slurry was volumetrically divided into 12 equal portions which were filtered, washed with dimethylformamide and dichloromethane and dried under vacuum. These 12 resin lots were given letter designations A through L for simplification for experimental description.

B: Reductive Amination

Each resin lot was reacted with the appropriate aldehyde or k tone as follows.

A	cyclohexanone
B	cyclohexane carboxaldehyde
C	benzaldehyde
D	phenylacetaldehyde
E	4'-biphenylcarboxaldehyde
F	No reductive amination.
G	2-Napthaldehyde
H	2-indanone
I	2-(N-Cbz)-ethanal
J	6-(N-Cbz)-hexanal
K	12-(N-Cbz)-dodecanal

Each resin lot was placed in a 50 mL solid phase reaction vessel, suspended in dimethylformamide (20 mL) and agitated for 30 minutes. The solvent was drained away and the resin was treated with 25 to 30 mL of dimethylformamide containing 3 equivalents (1.56 mmole) of aldehyde or ketone and 10 equivalents (327 mg) of sodiumcyanoborohydride. The reaction was agitated for 6 to 24 hours, and monitored by the Kaiser test. If incomplete reaction had occured the mixture was filtered away and a fresh solution of 25 to 30 mL of dimethylformamide containing 3 equivalents (1.56 mmole) of aldehyde or ketone and 10 equivalents (327 mg) of sodiumcyanoborohydride was added and reaction was continued for 6 to 24 hours. The reaction mixture was then filtered away and the resin mixtures were washed well with dimethylformamide and dichloromethane and dried under vacuum.

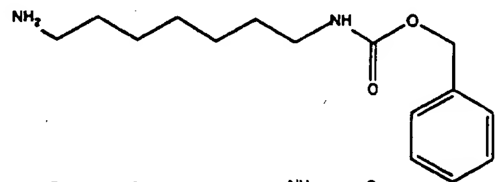
C. Bromoacetylation:

All lots A through L were bromoacetylated as follows. Each resin sample was placed in a 50 mL solid phase synthesis reaction vessel and suspended in DMF. The mixture was agitated for 30 minutes, and filtered away. The resin was then reacted with 12 equivalents bromoacetic

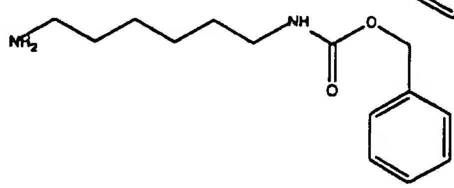
acid (867 mg) and 13.2 equivalents of diisopropylcarbodiimide (1.08 mL) in DMF (~ 10 mL / g resin). The reaction was agitated for 60 minutes, after which the solution was filtered away. The reaction vessel was charged with fresh bromoacetylation cocktail and the reaction was again agitated for 60 minutes. The solution was drained away and each lot was washed well with dimethylformamide followed by dichloromethane and dried under vacuum.

D. Amine Displacement Reactions

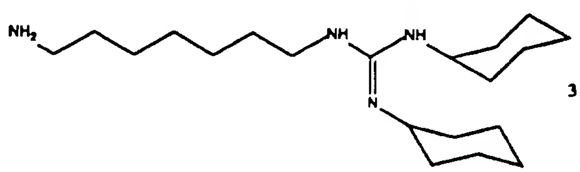
Amines #1 through #12 used in displacement reactions:



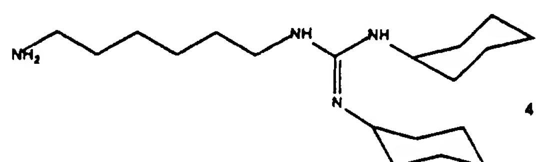
1



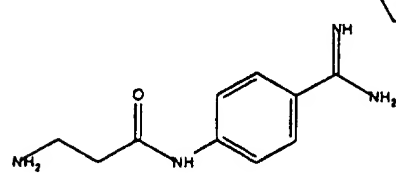
2



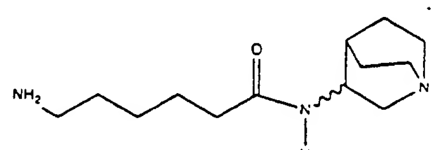
3



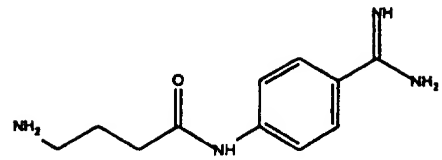
4



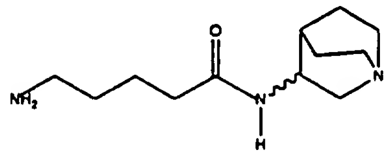
5



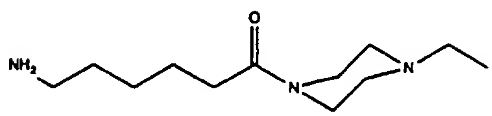
6



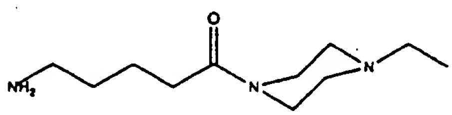
7



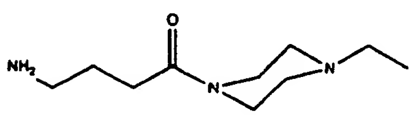
8



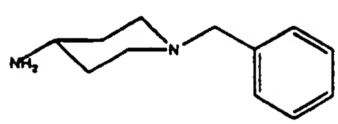
9



10



11



12

D. Amine Displacement Reactions

Each lettered lot of Resin (A through L) following bromoacetylation was again subdivided into 12 sub-lots and numbered sequentially i. e. (A1 through A12, B1 through B12, ... L1 through L12). Each sublot was reacted with the corresponding numbered amine as follows.

Amine #	Equivalents	Concentration in DMSO
1	20	0.43 M
2	20	0.43 M
3	9	0.193 M
4	13	0.284 M
5	14.5	0.315 M
6	14	0.302 M
7	20	0.43 M

8	23	0.50 M
9	23	0.50 M
10	23	0.50 M
11	17.5	0.38 M
12	20	0.43 M

Reactions were carried out in a specially designed multiple reaction device which is described in example 438. The 5 ml reaction vessles were pre-loaded with the appropriate amine solution in DMSO. Hydrochloride salts were treated with 2 equivalents of diisopropylethylamine *in situ* to provide free amino groups for the displacement reaction. The reactions were allowed to swirl for 15 hours or were sonicated at approximately 50 ^{o} C for 3 hours. The reaction flasks were then removed and the resin samples were washed by positive N_2 pressure using DMSO, DMF, dichloromethane, methanol, and dichloromethane.

E. Cyclization Reactions:

A fresh set of 12 5 mL reaction vessels charged with 3 mL of 2-butanol containing 2 M acetic acid are connected to the multiple reaction device so each resin sample is immersed in solution. The exposed gas dispersion tubes are connected to positive pressure nitrogen lines and cooled externally with dry ice. The 5 mL reaction vessels are placed in a 110 ^{o} C oil bath and heated for 48 to 90 hours. From time to time fresh butanol containing 2M acetic acid is added to maintain approximately 3 mL volume. After the reflux period the reaction flasks containing the now cleaved and cyclized piperzazine-diones are removed and the solvent removed by rotary evaporation. Completed piperazinedione mixtures are assayed by HPLC and laser desorption mass spectrometry, dissolved in a predetermined volume of DMSO and assayed for receptor binding or enzyme inhibition activity.

F. Further Reactions:

Piperazinedione mixtures derived from amines #1 and #2 are deprotected with hydrogen bromide in acetic acid as described in example 4, and can be acylated or guanidinated as described above or tested as free amines. All sublots were divided in half and reduced with lithium aluminum hydride as described in example 11.

Example 11. General Procedure for $LiAlH_4$ Reduction of Mixtures of Piperazinediones from Example 10.

The piperazinediones obtained from cyclization reactions or following protecting group removal, as residues in 5 mL flasks, are carefully treated with 1 M $LiAlH_4$ solution (20 equivalents) and attached to the multiple reaction device. In these instances, a plain glass column replaces the gas dispersion tube and positive nitrogen pressure is provided by plastic tubing attached to the glass column. External cooling of the condensor tubing is provided by packing the exposed glass and plastic tubing in dry ice. The 5 mL flasks are then placed in an 80 ^{o} C oil bath and the reactions refluxed 16 hours. The reactions are cooled to room temperature and poured into ice. The flasks are rinsed well sequentially with dichloromethane, methanol and water. The volatile organic solvents and water are removed on a speed-vac and the residue is extracted with acetonitrile (1.5 mL), evaporated and assayed or submitted to further modification such as acylation or guanidination.

Example 12. Bradykinin 2 Guinea Pig Ileum Receptor Binding Assay.

Guinea pig ileum membranes were preparation by Analytical Biological Services, Inc. Briefly, ilea were finely chopped and combined with TES buffer (25 mM, pH 6.8) containing 1 mM 1, 10-phenanthroline, 5 ug/ml soybean trypsin inhibitor, 100 ug/ml bacitracin, 1 mM benzamidine, and 100 uM phenylmethylsulfonyl fluoride. This mixture was homogenized in a Brinkman PT-20 Polytron (setting 7, 4 x 20 sec intervals) and subjected to differential centrifugation (1000 x g, 4^{0}C, 10 min). The pellet was discarded and the supernatant centrifuged for 15 min at 43,000 x g at 4^{0}C. The pellet was washed twice by resuspending in the buffer described above and

centrifuging as above. The pellet was resuspended and stored at -70^{0}C until use.

Guinea pig ileum membrane solution were incubated with ^{3}H-bradykinin (final concentration 0.3 nM) with or without test compounds in assay buffer (25 mM TES, pH 6.8 containing 1 mM 1,10 phenanthroline, 1 mM dithiothreitol, 2 uM captopril, 140 ug/ml bacitracin, and 0.1 % bovine serum albumin, BSA), at room temperature, for 45 minutes, at a final volume of 315 ul. All test compound dilutions were done in triplicate. Assays were harvested by quick filtration in a Tomtec Harvester 96, with ice-cold wash buffer consisting of 10 mM Tris/HCl, pH 7.5, 100 mM NaCl,.02% BSA, onto Wallec printed glassfiber Filtermat "B", which had been pre-soaked with 0.1% polyethyleneimine (PEI) and previously air-dried. Filtermats were counted in 9.5 ml Wallec Beta-Plate Scint, in Wallec 1450 MicroBeta Counter.

Example 13. Bradykinin 2 and Bradykinin 1 Human Receptor Clone Binding Assays.

Human bradykinin B_2 receptor was expressed in CHO-K1 (ATCC) cells. Preparation of membranes for binding assay was carried out by scraping cells from roller bottles in ice cold PBS and centrifuging at 1000 x g, at 4^{o}C for 15 minutes. The supernatant was discarded and pellet resuspended in Buffer A consisting of 25 mM TES(pH 6.8) with 2uM 1,10-phenanthroline, and centrifuged at 27,000 x g for 15 min. The pellet was washed once using the same buffer and centrifugation parameters. The final pellet was resuspended in Buffer B (Buffer A with 2uM captopril, 140 ug/ml bacitracin, 0.1% BSA), and stored in 1 ml aliquot, frozen at -70^{o}C until needed.

B_2 Binding assays were performed by incubating human clone membrane solution with ^{3}H-bradykinin (final concentration 0.3 nM) with or without test compounds in assay buffer (Buffer B with 1 mM dithiothreitol), at room temperature, for 45 minutes, at a final volume of 315 ul. All test compound dilutions were done in triplicate. Assays were harvested by quick filtration in a Tomtec Harvester 96, with ice-cold wash buffer consisting of 10 mM Tris/HCl, pH 7.5, 100 mM NaCl,.02% BSA, onto Wallec printed glassfiber Filtermat "B", which had been pre-soaked with 0.1% PEI

and previously air-dried. Filtermats were counted in 9.5 ml Wallec Beta-Plate Scint, in Wallec 1450 MicroBeta Counter.

Human lung fibroblasts IMR-90 cells were obtained from ATCC and propagated in DMEM media in 850 mm roller bottles until confluent. Three hours prior to harvesting, the cells were treated with interleukin 1-beta (200 pg/ml). After three hours, cells were scraped from roller bottles in ice cold PBS and centrifuging at 1000 x g, at 4^{o}C for 15 minutes. The supernatant was discarded and pellet resuspended in Buffer A consisting of 25 mM TES(pH 6.8) with 2 uM 1,10-phenanthroline, and centrifuged at 27,000 x g for 15 min. The pellet was washed once using the same buffer and centrifugation parameters. The final pellet was resuspended in Buffer B (Buffer A with 2 uM captopril, 140 ug/ml bacitracin, 0.1% BSA), and stored in 1 ml aliquot, frozen at -70^{o}C until use.

The human bradykinin B1 binding assays were performed by incubating IMR-90 membrane solution (approx. 150 ug/well) with ^{3}H-des-Arg^{10}Kallidin, (final concentration 0.5 nM) with or without test compounds in assay buffer (Buffer B with 1 mM dithiothreitol), at room temperature, for 45 minutes, at a final volume of 200 ul. All test compound dilutions were done in triplicate. Assays were harvested by quick filtration in a Tomtec Harvester 96, with ice-cold wash buffer consisting of 10 mM Tris/HCl, pH 7.5, 100 mM NaCl, 0.02% BSA, onto Wallec printed glassfiber Filtermat "B", which had been pre-soaked with 0.1% PEI and previously air-dried. Filtermats were counted in 9.5 ml Wallac Beta-Plate Scint, in Wallace 1450 MicroBeta Counter.

Example 14. Neurokinin 1 Human Receptor Clone Binding Assay.

The human NK-1 receptor was expressed in CHO-K1 (ATCC) cells. Preparation of membranes for binding assay was carried out by scraping cells from culture flasks in ice cold PBS and centrifuging at 500 x g, at 4^{o}C for 10 minutes. The supernatant was discarded and pellet resuspended in 50 mM Tris/HCl, pH 7.4 with 1 mM 1,10 phenanthroline, and centrifuged at 27,000 x g for 15 min. The pellet was washed once using 20 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 30 mM KCl, 0.02% BSA and 100 uM thiorphan and centrifuged as above. The final pellet was resuspended in the wash buffer (above) and stored in 1 ml aliquot, frozen at -70^{o}C until needed.

Human NK1 receptor binding assays were performed by incubating human clone membrane solution (50 ug/well in 125 ul) with ^{3}H-substance P (final concentration 1.0 nM) with or without test compounds in assay buffer (Buffer B with 1 mM dithiothreitol), at room temperature, for 30 minutes, at a final volume of 315 ul. All test compound dilutions were done in triplicate. Assays were harvested by quick filtration in a Tomtec Harvester 96, with ice-cold wash buffer consisting of 20 mM Tris/HCl, pH 7.5, 120 mM NaCl, 5 mM KCl and 0.02% BSA, onto Wallec printed glassfiber Filtermat "B", which had been pre-soaked with 0.1% PEI and previously air-dried. Filtermats were counted in 9.5 ml Wallec Beta-Plate Scint, in Wallec 1450 MicroBeta Counter.

Example 15. Neurokinin 2 Human Receptor Clone Binding Assay.

The cell membrane preparation and receptor binding procedure are identical to that described above for the NK-1 receptor (Example 14) except that ^{3}H-neurokinin A was used as the ligand at 2.0 nM.

Example 16. Mu-Opioid Human Receptor Clone Binding Assay.

The human mu-opioid receptor was expressed in CHO-K1 (ATCC) cells. Preparation of membranes for binding assay was carried out by scraping cells from culture flasks in ice cold PBS and centrifuging at 500 x g, at 4^{o}C for 10 minutes. The supernatant was discarded and pellet resuspended in 10 mM Tris/HCl, pH 7.4 with 0.32 M sucrose and centrifuged for 30 min at 40C at 27,000 x g. The supernatant was discarded and the pellet resuspended in the wash buffer (above) and stored in 1 ml aliquot, frozen at -70^{o}C until needed.

Human mu-opioid binding assays were performed by incubating human clone membrane solution (50 ug/well in 125 ul) with ^{3}H-DAMGO ([D-Ala^2, N-Me-Phe^4, Gly^5-ol]enkephalin; final concentration 5.0 nM) with or without test compounds in assay buffer (Buffer B with 1 mM dithiothreitol), at room temperature, for 60 minutes, at a final volume of 315 ul. All test compound dilutions were done in triplicate. Assays were harvested by quick filtration in a Tomtec Harvester 96, with ice-cold wash buffer consisting of 50 mM Tris/HCl, pH 7.4 onto Wallec printed glassfiber

Filtermat "B", which had been pre-soaked with 0.1% PEI and previously air-dried. Filtermats were counted in 9.5 ml Wallec Beta-Plate Scint, in Wallec 1450 MicroBeta Counter.

Example 17. k-Opioid Guinea Pig Brain Receptor Binding Assay.

Preparation of guinea pig brain membrane for Kappa binding assay was carried out by homogenizing tissue with 5 second bursts of tissue homogenizer, until smooth, in ice-cold assay buffer consisting of 10 mM Tris/HCl, pH 7.4 with 0.32 M Sucrose. The homogenate was centrifuged at 1,500 x g, for 10 minutes at 4°C. The pellet was discarded and supernatant was centrifuged at 30,000 x g, for 30 min at 4°C. Supernatant was discarded and pellet resuspended in fresh assay buffer, and in 1 ml aliquot, frozen at -70°C until needed. Protein concentration was determined by Bradford analysis.

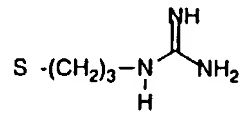
k-Opioid receptro binding assays were performed by incubating guinea pig brain membrane solution (50 ug/well in 125 ul final concentration) with ^{3}H-U69,593 (final concentration 5.0 nM, New England Nuclear) with or without test compounds in assay buffer , at room temperature, for 60 minutes, at a final volume of 315 ul. All test compound dilutions were done in triplicate. To block mu receptor sites, 0.1 uM solution of unlabeled DAMGO, in assay buffer, was added to all wells. Assays were harvested by quick filtration in a Tomtec Harvester 96, with ice-cold wash buffer consisting of 50 mM Tris/HCl, pH 7.4, onto Wallec printed glassfiber Filtermat "B", which had been pre-soaked with 0.1% PEI and previously air-dried. Filtermats were counted in 9.5 ml Wallec Beta-Plate Scint, in Wallec 1450 MicroBeta Counter.

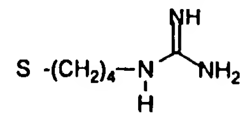
Example 18. Bradykinin Induced Calcium Flux in Human WI38 Fibroblasts.

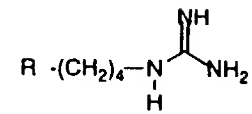
A fluorescent indicator (Fura-2) method was used to measure cytosolic Ca(+2) (A. Pilyavskaya et al. FASEB J. 9, 6, A1371, 1995). Human lung fibroblasts are loaded with Fura-2 and, after incubation with BK in the absence and presence of antagonists, the cells are analyzed in a Perkin-Elmer LS50B spectrofluorometer using excitation wavelengths of 340 & 380

nm and an emission wavelength of 510 nm. Cells are maintained in suspension during the measurements.

Examples 19 - 39. Specific Piperazines and Piperazinediones Prepared According to Method B.

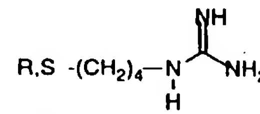
R6A = 

R6B = 

R6C = 

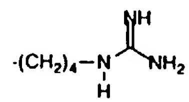
R6D = S $-(CH_2)_3-NH_2$

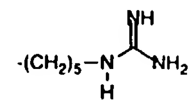
R6E = R,S $-(CH_2)_4-NH_2$

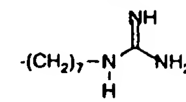
R6F = 

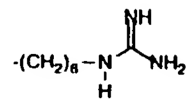
Example	X	R^5	R^7	R^6	HPLC [a]	Mass Spec. (M+H)	CP
19	O	R-benzyl	$-CH_2-C_6H_{11}$	R6A	20.0	400	2006
20	H_2	R-benzyl	$-CH_2-C_6H_{11}$	R6D	17.8	343	2011
21	H_2	R-benzyl	$-CH_2-C_6H_{11}$	R6A	19.6	386	2012
22	H_2	R-benzyl	$-C_6H_{11}$	R6B	17.3	372	2029
23	H_2	R-benzyl	benzyl	R6B	18.1	380	2034
24	H_2	R-benzyl	2-indanyl	R6B	21.2	406	2035
25	H_2	S-benzyl	$-C_6H_{11}$	R6B	16.5	372	2051
26	H_2	S-benzyl	$-CH_2-C_6H_{11}$	R6B	19.7	386	2052
27	H_2	S-benzyl	benzyl	R6B	19.5	380	2053
28	H_2	S-benzyl	$-CH_2CH_2Ph$	R6B	19.5	393	2054
29	H_2	S-benzyl	$-C_6H_{11}$	R6C	17.0	372	2075
30	H_2	S-benzyl	$-CH_2-C_6H_{11}$	R6C	17.3	386	2076
31	H_2	S-benzyl	benzyl	R6C	17.8	380	2077
32	H_2	S-benzyl	$-CH_2CH_2Ph$	R6C	19.1	393	2078
33	H_2	R-benzyl	$-CH_2CH_2Ph$	R6B	18.3	394	2080
34	H_2	R-benzyl	$-CH_2-C_6H_{11}$	R6B	18.7	386	2081
35	H_2	R-benzyl	$CH_2-C_6H_{11}$	R6C	17.6	386	2082
36	H_2	R-benzyl	$-CH_2CH_2Ph$	R6C	18.0	394	2083
37	H_2	R,S-2-indanyl	3,4 Di-MeOBn	R6E	17-18	425	2102
38	H_2	R,S-2-indanyl	$-CH_2-C_6H_{11}$	R6F	20.3/22.1	412	2103
39	H_2	R,S,-benzyl	3,4 Di-MeOBn	R6F	17.4/18.0	440	2104

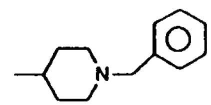
Examples 40 - 61 . Specific Piperazines and Piperazihediones Prepared According to Method A.

S13 = 

S14 = 

S1 = 

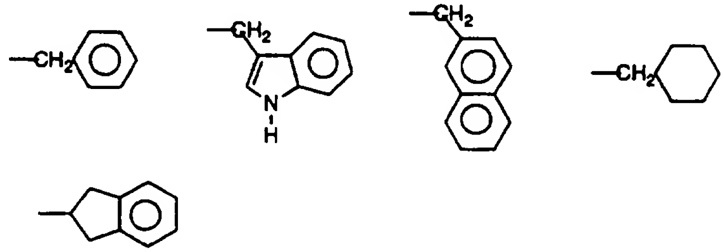
S2 = 

S12 = 

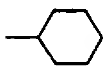
CP#	Example	X	R1	R2	R3	HPLC	MS (M+H)
2114	40	H2	R,S-benzyl	-CH_2-C_6H_{11}	S13	19.9	386
2115	41	H2	R,S-benzyl	-CH_2-C_6H_{11}	S14	19.8	400
2116	42	H2	R,S-benzyl	-CH_2-C_6H_{11}	S2	19.5	414
2117	43	H2	R,S-benzyl	-CH_2-C_6H_{11}	S1	20.2	428
2151	44	O	S-benzyl	H	S1	16.7	360
2371	45	O	S-3-indole	-CH_2CH_2Ph	S12	24.1	521
2372	46	O	R-3-indole	-CH_2CH_2Ph	S12	24.1	521
2373	47	O	S-benzyl	-CH_2CH_2Ph	S12	24.0	482
2374	48	O	R-benzyl	-CH_2CH_2Ph	S12	24.0	482
2399	49	O	S -CH_2-C_6H_{11}	-CH_2CH_2Ph	S12	29.0	488
2400	50	O	R -CH_2-C_6H_{11}	-CH_2CH_2Ph	S12	29.0	488
2425	51	O	S -CH_2-2-Nph	-CH_2CH_2Ph	S12	27.3	532
2426	52	O	R -CH_2-2-Nph	-CH_2CH_2Ph	S12	27.5	532
2427	53	O	H	-CH_2CH_2Ph	S12	18.4	392
2428	54	O	R,S-2-indanyl	-CH_2CH_2Ph	S12	26.5	508
2429	55	O	S -CH_2-2-Nph	-$CH_2C_6H_{11}$	S12	29.1	524
2430	56	O	R -CH_2-2-Nph	-$CH_2C_6H_{11}$	S12	29.2	524
2431	57	O	R,S-2-indanyl	-$CH_2C_6H_{11}$	S12	29.1	500
2456	58	O	S -CH_2-C_6H_{11}	$CH_2C_6H_{11}$	S12	31.2	480
2457	59	O	R -CH_2-C_6H_{11}	$CH_2C_6H_{11}$	S12	31.3	480
2458	60	O	R,S-2-indanyl	CH_2-2-Nph	S2	28.1	512
2459	61	O	R,S-2-indanyl	4'-biphenyl	S1	31.0	552

Examples 62 through 437. Combinat rial Mixtures of Piperazindi nes and Piperazines Prepared According to Methods 4,6, 10, 11.

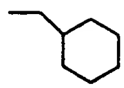
R^2 = M =



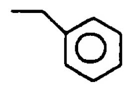
A =



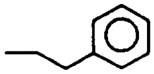
B =



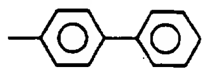
C =



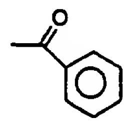
D =



E =

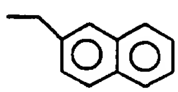


F1 =

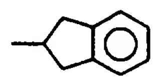


F2 = H

G =



H =

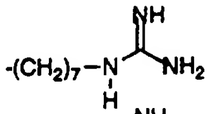


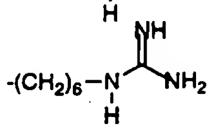
I = $-(CH_2)_2-NH-C(=NH)-NH_2$ $\quad$ I_{NH2} = $-(CH_2)_2-NH_2$ $\quad$ I_{Cbz} = $-(CH_2)_2-NHCbz$

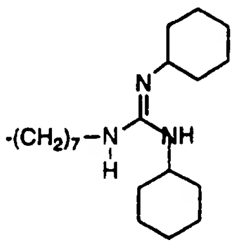
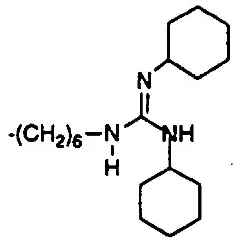
J = $-(CH_2)_6-NH-C(=NH)-NH_2$ $\quad$ J_{NH2} = $-(CH_2)_6-NH_2$ $\quad$ J_{Cbz} = $-(CH_2)_6-NHCbz$

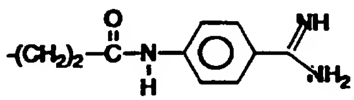
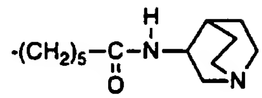
K = $-(CH_2)_{12}-NH-C(=NH)-NH_2$ $\quad$ K_{NH2} = $-(CH_2)_{12}-NH_2$ $\quad$ K_{Cbz} = $-(CH_2)_{12}-NHCbz$

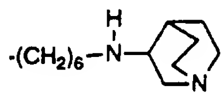
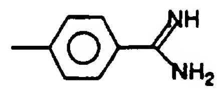
L = $-C(=O)-(CH_2)_{10}-CH_3$

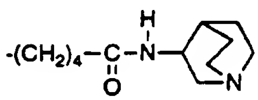
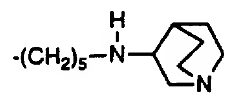
S1 =  **S1R** = $-(CH_2)_7-NH_2$ **S1Cbz** = $-(CH_2)_7-NH\text{-}Cbz$

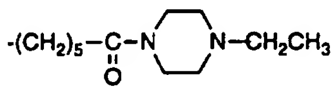
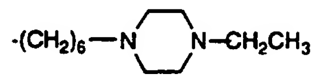
S2 =  **S2R** = $-(CH_2)_6-NH_2$ **S2Cbz** = $-(CH_2)_6-NH\text{-}Cbz$

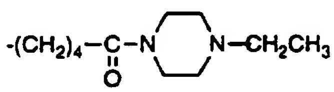
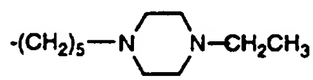
S3 =  **S4** = 

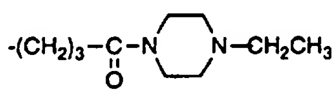
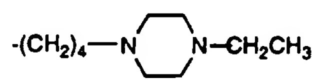
S5 =  **S6** = 

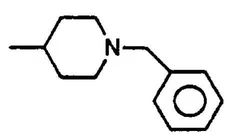
S6R =  **S7** = 

S8 =  **S8R** = 

S9 =  **S9R** = 

S10 =  **S10R** = 

S11 =  **S11R** = 

S12 = 

Example	X	R1	R2	R3
62	O	A	M	S1
63	O	A	M	S2
64	O	A	M	S1R
65	O	A	M	S2R
66	O	A	M	S3
67	O	A	M	S4
68	O	A	M	S5
69	O	A	M	S6
70	O	A	M	S7
71	O	A	M	S8
72	O	A	M	S9
73	O	A	M	S10
74	O	A	M	S11
75	O	A	M	S12
76	O	A	M	S1Cbz

77	O	A	M	S2Cbz
78	H_2	A	M	S1R
79	H_2	A	M	S2R
80	H_2	A	M	S6R
81	H_2	A	M	S8R
82	H_2	A	M	S9R
83	H_2	A	M	S10R
84	H_2	A	M	S11R
85	H_2	A	M	S12
86	H_2	A	M	S1
87	H_2	A	M	S2

Example	X	R1	R2	R3
88	O	B	M	S1
89	O	B	M	S2
90	O	B	M	S1R
91	O	B	M	S2R
92	O	B	M	S3
93	O	B	M	S4
94	O	B	M	S5
95	O	B	M	S6
96	O	B	M	S7
97	O	B	M	S8
98	O	B	M	S9
99	O	B	M	S10
100	O	B	M	S11
101	O	B	M	S12
102	O	B	M	S1Cbz
103	O	B	M	S2Cbz
104	H_2	B	M	S1R
105	H_2	B	M	S2R
106	H_2	B	M	S6R
107	H_2	B	M	S8R
108	H_2	B	M	S9R
109	H_2	B	M	S10R
110	H_2	B	M	S11R
111	H_2	B	M	S12
112	H_2	B	M	S1
113	H_2	B	M	S2

Example	X	R1	R2	R3
114	O	C	M	S1
115	O	C	M	S2
116	O	C	M	S1R
117	O	C	M	S2R
118	O	C	M	S3
119	O	C	M	S4
120	O	C	M	S5
121	O	C	M	S6
122	O	C	M	S7
123	O	C	M	S8
124	O	C	M	S9
125	O	C	M	S10
126	O	C	M	S11
127	O	C	M	S12
128	O	C	M	S1Cbz
129	O	C	M	S2Cbz
130	H_2	C	M	S1R
131	H_2	C	M	S2R
132	H_2	C	M	S6R
133	H_2	C	M	S8R
134	H_2	C	M	S9R

135	H_2	C	M	S10R
136	H_2	C	M	S11R
137	H_2	C	M	S12
138	H_2	C	M	S1
139	H_2	C	M	S2

Example	X	R1	R2	R3
140	O	D	M	S1
141	O	D	M	S2
142	O	D	M	S1R
143	O	D	M	S2R
144	O	D	M	S3
145	O	D	M	S4
146	O	D	M	S5
147	O	D	M	S6
148	O	D	M	S7
149	O	D	M	S8
150	O	D	M	S9
151	O	D	M	S10
152	O	D	M	S11
153	O	D	M	S12
154	O	D	M	S1Cbz
155	O	D	M	S2Cbz
156	H_2	D	M	S1R
157	H_2	D	M	S2R
158	H_2	D	M	S6R
159	H_2	D	M	S8R
160	H_2	D	M	S9R
161	H_2	D	M	S10R
162	H_2	D	M	S11R
163	H_2	D	M	S12
164	H_2	D	M	S1
165	H_2	D	M	S2

Example	X	R1	R2	R3
166	O	E	M	S1
167	O	E	M	S2
168	O	E	M	S1R
169	O	E	M	S2R
170	O	E	M	S3
171	O	E	M	S4
172	O	E	M	S5
173	O	E	M	S6
174	O	E	M	S7
175	O	E	M	S8
176	O	E	M	S9
177	O	E	M	S10
178	O	E	M	S11
179	O	E	M	S12
180	O	E	M	S1Cbz
181	O	E	M	S2Cbz
182	H_2	E	M	S1R
183	H_2	E	M	S2R
184	H_2	E	M	S6R
185	H_2	E	M	S8R
186	H_2	E	M	S9R
187	H_2	E	M	S10R
188	H_2	E	M	S11R
189	H_2	E	M	S12
190	H_2	E	M	S1
191	H_2	E	M	S2

Example	X	R1	R2	R3
192	O	F2	M	S1
193	O	F2	M	S2
194	O	F2	M	S1R
195	O	F2	M	S2R
196	O	F2	M	S3

197	O	F2	M	S4
198	O	F2	M	S5
199	O	F2	M	S6
200	O	F2	M	S7
201	O	F2	M	S8
202	O	F2	M	S9
203	O	F2	M	S10
204	O	F2	M	S11
205	O	F2	M	S12
206	O	F2	M	S1Cbz
207	O	F2	M	S2Cbz
208	H_2	F2	M	S1R
209	H_2	F2	M	S2R
210	H_2	F2	M	S6R
211	H_2	F2	M	S8R
212	H_2	F2	M	S9R
213	H_2	F2	M	S10R
214	H_2	F2	M	S11R
215	H_2	F2	M	S12
216	H_2	F2	M	S1
217	H_2	F2	M	S2

Example	X	R1	R2	R3
218	O	G	M	S1
219	O	G	M	S2
220	O	G	M	S1R
221	O	G	M	S2R
222	O	G	M	S3
223	O	G	M	S4
224	O	G	M	S5
225	O	G	M	S6
226	O	G	M	S7
227	O	G	M	S8
228	O	G	M	S9
229	O	G	M	S10
230	O	G	M	S11
231	O	G	M	S12
232	O	G	M	S1Cbz
233	O	G	M	S2Cbz
234	H_2	G	M	S1R
235	H_2	G	M	S2R
236	H_2	G	M	S6R
237	H_2	G	M	S8R
238	H_2	G	M	S9R
239	H_2	G	M	S10R
240	H_2	G	M	S11R
241	H_2	G	M	S12
242	H_2	G	M	S1
243	H_2	G	M	S2

Example	X	R1	R2	R3
244	O	H	M	S1
245	O	H	M	S2
246	O	H	M	S1R
247	O	H	M	S2R
248	O	H	M	S3
249	O	H	M	S4
250	O	H	M	S5
251	O	H	M	S6
252	O	H	M	S7
253	O	H	M	S8
254	O	H	M	S9
255	O	H	M	S10
256	O	H	M	S11
257	O	H	M	S12
258	O	H	M	S1Cbz
259	O	H	M	S2Cbz
260	H_2	H	M	S1R
261	H_2	H	M	S2R

263	H_2	H	M	S6R
264	H_2	H	M	S8R
265	H_2	H	M	S9R
266	H_2	H	M	S10R
267	H_2	H	M	S11R
268	H_2	H	M	S12
269	H_2	H	M	S1
270	H_2	H	M	S2

Example	X	R1	R2	R3
271	O	I	M	S1
272	O	I	M	S2
273	O	I	M	S1R
274	O	I	M	S2R
275	O	I	M	S3
276	O	I	M	S4
277	O	I	M	S5
278	O	I	M	S6
279	O	I	M	S7
280	O	I	M	S8
281	O	I	M	S9
282	O	I	M	S10
283	O	I	M	S11
284	O	I	M	S12
285	O	I	M	S1Cbz
286	O	I	M	S2Cbz
287	H_2	I	M	S1R
288	H_2	I	M	S2R
289	H_2	I	M	S6R
290	H_2	I	M	S8R
291	H_2	I	M	S9R
292	H_2	I	M	S10R
293	H_2	I	M	S11R
294	H_2	I	M	S12
295	H_2	I	M	S1
296	H_2	I	M	S2

Example	X	R1	R2	R3
297	O	J	M	S1
298	O	J	M	S2
299	O	J	M	S1R
300	O	J	M	S2R
301	O	J	M	S3
302	O	J	M	S4
303	O	J	M	S5
304	O	J	M	S6
305	O	J	M	S7
306	O	J	M	S8
307	O	J	M	S9
308	O	J	M	S10
309	O	J	M	S11
310	O	J	M	S12
311	O	J	M	S1Cbz
312	O	J	M	S2Cbz
313	H_2	J	M	S1R
314	H_2	J	M	S2R
315	H_2	J	M	S6R
316	H_2	J	M	S8R
317	H_2	J	M	S9R
318	H_2	J	M	S10R
319	H_2	J	M	S11R
320	H_2	J	M	S12
321	H_2	J	M	S1
322	H_2	J	M	S2

Example	X	R1	R2	R3
323	O	K	M	S1
324	O	K	M	S2

325	O	K	M	S1R
326	O	K	M	S2R
327	O	K	M	S3
328	O	K	M	S4
329	O	K	M	S5
330	O	K	M	S6
331	O	K	M	S7
332	O	K	M	S8
333	O	K	M	S9
334	O	K	M	S10
335	O	K	M	S11
336	O	K	M	S12
337	O	K	M	S1Cbz
338	O	K	M	S2Cbz
339	H_2	K	M	S1R
340	H_2	K	M	S2R
341	H_2	K	M	S6R
342	H_2	K	M	S8R
343	H_2	K	M	S9R
344	H_2	K	M	S10R
345	H_2	K	M	S11R
346	H_2	K	M	S12
347	H_2	K	M	S1
348	H_2	K	M	S2
349	O	K_{NH2}	M	S1R
350	O	K_{NH2}	M	S2R
351	O	K_{NH2}	M	S3
352	O	K_{NH2}	M	S4
353	O	K_{NH2}	M	S5
354	O	K_{NH2}	M	S6
355	O	K_{NH2}	M	S7
356	O	K_{NH2}	M	S8
357	O	K_{NH2}	M	S9
358	O	K_{NH2}	M	S10
359	O	K_{NH2}	M	S11
360	O	K_{NH2}	M	S12
361	O	K_{CBZ}	M	S3
362	O	K_{CBZ}	M	S4
363	O	K_{CBZ}	M	S5
364	O	K_{CBZ}	M	S6
365	O	K_{CBZ}	M	S7
366	O	K_{CBZ}	M	S8
367	O	K_{CBZ}	M	S9
368	O	K_{CBZ}	M	S10
369	O	K_{CBZ}	M	S11
370	O	K_{CBZ}	M	S12
371	O	K_{CBZ}	M	S1Cbz
372	O	K_{CBZ}	M	S2Cbz
373	O	I_{NH2}	M	S1R
374	O	I_{NH2}	M	S2R
375	O	I_{NH2}	M	S3
376	O	I_{NH2}	M	S4
377	O	I_{NH2}	M	S5
378	O	I_{NH2}	M	S6
379	O	I_{NH2}	M	S7
380	O	I_{NH2}	M	S8
381	O	I_{NH2}	M	S9
382	O	I_{NH2}	M	S10
384	O	I_{NH2}	M	S11

385	O	I_{NH2}	M	S12
386	O	I_{Cbz}	M	S3
387	O	I_{Cbz}	M	S4
388	O	I_{Cbz}	M	S5
389	O	I_{Cbz}	M	S6
390	O	I_{Cbz}	M	S7
391	O	I_{Cbz}	M	S8
392	O	I_{Cbz}	M	S9
393	O	I_{Cbz}	M	S10
394	O	I_{Cbz}	M	S11
395	O	I_{Cbz}	M	S12
396	O	I_{Cbz}	M	S1Cbz
397	O	I_{Cbz}	M	S2Cbz
398	O	J_{NH2}	M	S1R
399	O	J_{NH2}	M	S2R
400	O	J_{NH2}	M	S3
401	O	J_{NH2}	M	S4
402	O	J_{NH2}	M	S5
403	O	J_{NH2}	M	S6
404	O	J_{NH2}	M	S7
405	O	J_{NH2}	M	S8
406	O	J_{NH2}	M	S9
407	O	J_{NH2}	M	S10
408	O	J_{NH2}	M	S11
409	O	J_{NH2}	M	S12
410	O	J_{Cbz}	M	S3
411	O	J_{Cbz}	M	S4
412	O	J_{Cbz}	M	S5
413	O	J_{Cbz}	M	S6
414	O	J_{Cbz}	M	S7
415	O	J_{Cbz}	M	S8
416	O	J_{Cbz}	M	S9
417	O	J_{Cbz}	M	S10
418	O	J_{Cbz}	M	S11
419	O	J_{Cbz}	M	S12
420	O	J_{Cbz}	M	S1Cbz
421	O	J_{Cbz}	M	S2Cbz
422	H_2	F1	M	S6R
423	H_2	F1	M	S8R
424	H_2	F1	M	S9R
425	H_2	F1	M	S10R
426	H_2	F1	M	S11R
427	H_2	F1	M	S12
428	H_2	F1	M	S1
429	H_2	F1	M	S2
430	H_2	L	M	S6R
431	H_2	L	M	S8R
432	H_2	L	M	S9R
433	H_2	L	M	S10R
434	H_2	L	M	S11R
435	H_2	L	M	S12
436	H_2	L	M	S1
437	H_2	L	M	S2

Example 438. Description f the Multiple Reaction Device.

The multiple solid phase reaction device consists of polypropylene block clamps which may hold 1 to 12 or more mini-reactors. The mini-reactors consist of 5 mL round bottom flasks (Ace glass model 9592-04) with ground glass joints, and screw adapters, the resin is introduced into a gas dispersion tube (7 mm outside diameter, Ace glass model 9436) with a medium porosity glass fritt. The gas dispersion tube is held in an air tight fit with a ground glass joint screw adapter (Ace model 5028-25). The top of the gas dispersion tube may be fitted to plastic tubing carring nitrogen, solvents or liquid or gaseous reagents. Cooling to allow solvent reflux can be obtained by packing the fixed dispersion tubes in an insulated container of dry-ice. The reaction vessels may be sonicated, swirled on an orbital table, cooled in baths or heated in a high-temperature oil bath. Individual reactors can be opened, heated, or cooled, or left idle, on an individual basis as the need may arise, unlike block reactors described by DeWitt et al.

<u>WE CLAIM:</u>

1. A method of synthesizing 1,4-piperazines and derivatives thereof comprising simultaneously cyclizing and cleaving an optionally substituted glycyl residue or dipeptoid from a polymeric support.

2. The method according to claim 1 wherein said cyclization and cleavage is accomplished via heating in a non-reactive solvent in the presence of a catalyst.

3. The method according to claim 1 wherein said solvent is 2-butanol and said catalyst is acetic acid.

4. The method of synthesizing 1,4-piperazine-2,5-diones according to claim 1 further comprising, prior to cylization and cleavage,

attaching an N-protected α-amino acid to a polymeric support;

deprotecting said α-amino acid; reductively alkylating said α-amino acid with an aldehyde or ketone; bromoacetylating resulting secondary amine; and

reacting resulting bromoacetyl compound with a primary amine.

5. The method according to claim 4 wherein said α-amino acid is α-carbon substituted with benzyl, substituted benzyl, indole, methylnaphthyl, cyclohexyl, methylcyclohexyl, indanyl, (C1-C5) alkyloxyaryl, aminoalkyl (C1-C8), guanidino alkyl (C1-C8), alkylcarboxylic, hydroxyalkyl (C1-C5), alkylthioalkyl, (C1-C3) alkylbenzamidio or hydrogen.

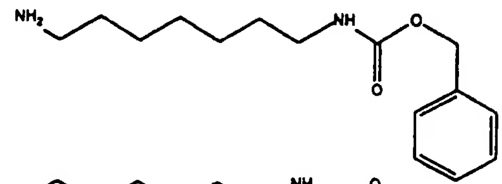
6. The method according to claim 4 wherein said α-amino acid is selected from from L- or D-Phenylanine, D- or L-Tryptophan, D- or l-Cyclohexylalanine, D- or L- ß-2-Naphthylalanine, and D- or L-Indanylglycine.

7. The method according to claim 4 wherein said aldehyde is of the formula R1C(O)H, wherein R1 is cyclohexyl, methylcyclohexyl, benzyl, substituted benzyl, phenethyl, biphenyl, benzoyl, hydrogen, methylnapthyl, indanyl, guanidinoalkyl (C2-C8), aminoalkyl (C2-C8), arylalkylcarbamate with alkyl chains of C2 to C8, alkanoyl (C1-C12) or alkyl (C1-C12).

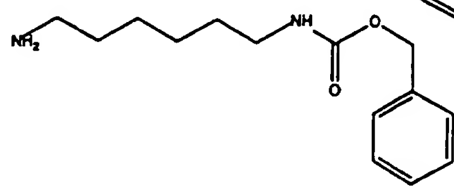
8. The method according to claim 3 wherein said aldehyde or ketone group is selected from cyclohexanone, cyclohexanone carboxaldehyde, benzaldehyde, phenylacetalaldehyde, 4'-biphenylcarboxaldehyde, 2-Napthaldehyde, 2-indanone-, 2-(N-Cbz)-ethanal, 6-(N-Cbz)-hexanal and 12-(N-Cbz)-dodecanal.

9. The method according to claim 4 wherein said primary amine is of the formula H2NR3 where R3 is selected from cyclohexyl, methylcyclohexyl, benzyl, substituted benzyl, phenethyl, biphenyl, benzoyl, hydrogen, methylnapthyl, indanyl, guanidinoalkyl (C2-C8), aminoalkyl (C2-C8), arylalkylcarbamates with alkyl chains of C2 to C8, alkanoyl (C1-C12), alkyl (C1-C12), (C2-C12)-alkylguanidinoalkyl (-C2-C8), benzamidino, (C1-C6) alkylbenzamidino, (C1-C6) alkylquinuclidine, (C1-C6) alkylpiperazine, C1 to C6 alkyl N-substituted piperazine, (C1-C6) alkylamino piperidine, (C1-C6) alkylamino N-substituted piperidine, (C1-C6) alkylcarboxyamidobenzamidino, (C1-C6) alkylcarboxamidoquinuclidine, (C1-C6) alkylcarboxamido piperazine, C1 to C6 alkylcarboxyl N-substituted piperazine, (C1-C6) alkylcarboxamidino piperidine and (C1-C6) alkylcarboxamidino N-substituted piperidine.

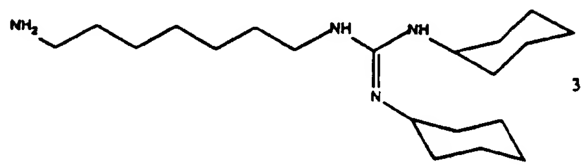
10. The method according to claim 4 wherein said primary amine is selected from



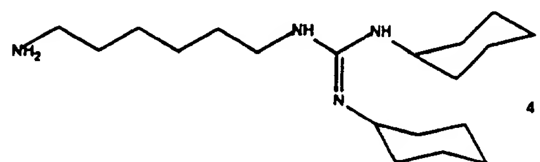
1



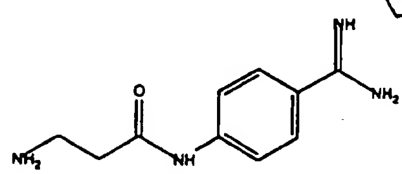
2



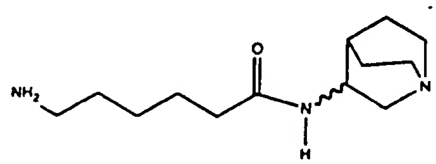
3



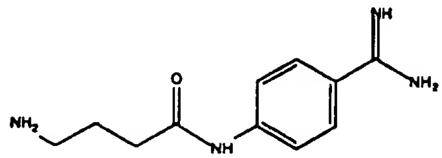
4



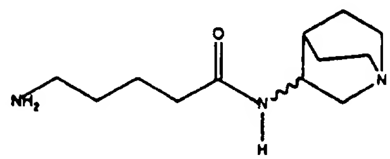
5



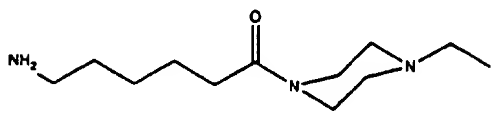
6



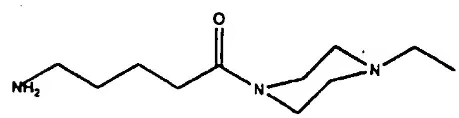
7



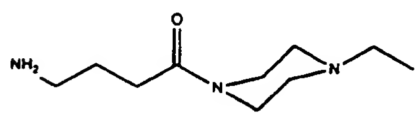
8



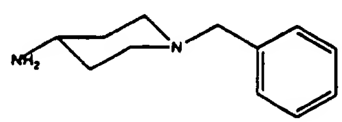
9



10



11



12

11. The method of synthesizing piperazines according to claim 1 further comprising reducing resulting 1,4-piperazine-2,5-dione.

12. The method according to claim 10 wherein said 1,4-piperazine-2,5-dione is reduced using diborane or lithium aluminum hydride.

13. The method of synthesizing 1,4-piperazine-2,5-diones according to claim 1 further comprising, prior to cyclization and cleavage,

attaching an N-protected α-amino acid to a polymeric support;

deprotecting said α-amino acid; coupling second N-protected α-amino acid residue to attached α-amino acid; deprotecting said residue; and

reductively alkylating resulting dipeptoid with an aldehyde or ketone group.

14. The method according to claim 13 wherein said first α-amino acid is α-carbon substituted with benzyl, substituted benzyl, indole, methylnaphthyl, cyclohexyl, methylcyclohexyl, indanyl, (C1-C5) alkyloxyaryl, aminoalkyl (C1-C8), guanidino alkyl (C1-C8), alkylcarboxylic, hydroxyalkyl (C1-C5), alkylthioalkyl, (C1-C3) alkylbenzamidio or hydrogen.

15. The method according to claim 15 wherein said second α-amino acid is α-carbon substituted with cyclohexyl, methylcyclohexyl, benzyl, substituted benzyl, phenethyl, biphenyl, benzoyl, hydrogen, methylnapthyl, indanyl, guanidinoalkyl (C2-C8), aminoalkyl (C2-C8), arylalkylcarbamates with alkyl chains of C2 to C8, alkanoyl (C1-C12), alkyl (C1-C12), (C2-C12)-alkylguanidinoalkyl (-C2-C8), benzamidino, (C1-C6) alkylbenzamidino, (C1-C6) alkylquinuclidine, (C1-C6) alkylpiperazine, C1 to C6 alkyl N-substituted piperazine, (C1-C6) alkylamino piperidine, (C1-C6) alkylamino N-substituted piperidine, (C1-C6) alkylcarboxyamidobenzamidino, (C1-C6) alkylcarboxamidoquinuclidine, (C1-C6) alkylcarboxamido piperazine, C1 to C6 alkylcarboxyl N-substituted piperazine, (C1-C6) alkylcarboxamidino piperidine or (C1-C6) alkylcarboxamidino N-substituted piperidine.

16. The method according to claim 13 wherein said α-amino acids are independently selected from L- or D-Phenylanine, D- or L-Tryptophan, D- or l- Cyclohexylalanine, D- or L- ß-2-Naphthylalanine, and D- or L-Indanylglycine.

17. The method according to claim 13 wherein said aldehyde is of the formula R7C(O)H, where R7 is cyclohexyl, methylcyclohexyl, benzyl, substituted benzyl, phenethyl, biphenyl, benzoyl, hydrogen, methylnapthyl,

indanyl, guanidinoalkyl (C2-C8), aminoalkyl (C2-C8), arylalkylcarbamate with alkyl chains of C2 to C8, alkanoyl (C1-C12), or alkyl (C1-C12).

18. The method according to claim 13 wherein said aldehyde or ketone is selected from cyclohexanone, cyclohexanone carboxaldehyde, benzaldehyde, phenylacetalaldehyde, 4'-biphenylcarboxaldehyde, 2-Napthaldehyde, 2-indanone-, 2-(N-Cbz)-ethanal, 6-(N-Cbz)-hexanal, and 12-(N-Cbz)-dodecanal.

19. A method of synthesizing [a combinatorial library of] optionally substituted piperazine derivatives comprising simultaneously cyclizing and cleaving optionally substituted glycyl residues from a polymeric support.

20. A method of synthesizing (W)(X)(Z) N-substituted1,4-piperazine-2,5-dione derivatives according to claim 19, further comprising, prior to cyclization and cleavage,

attaching W N-protected α-amino acids to W polymeric support resins, where W is the number of chosen α-amino acids;

mixing 1/W of each resin into W mixtures, each mixture having an equivalent amount of each resin;

dividing said mixtures into X aliquots, where X is the number of chosen aldehyde or ketone groups;

deprotecting said α-amino acids;

reductively alkylating said α-amino acids in each (X)(W) aliquot with a different aldehyde or ketone group, where (X)(W) is the total number of aliquots;

bromoacetylating resulting secondary amines;

dividing each (X)(W) aliquots into Z aliquots, where Z is the number of chosen primary amines; and

reacting each (X)(W)(Z) aliquot with a different primary amine, where (X)(W)(Z) is the total number of aliquots reacted with amines.

21. The method according to claim 20 further comprising dividing said (X)(W)(Z) aliquots of derivatized piperazinediones into two equal portions, Z1 and Z2; and reducing derivatized piperazinediones of Z1 aliquots to piperazine derivatives.

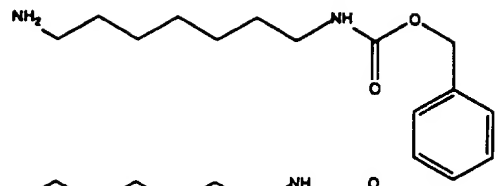
22. The method according to claim 20 wherein α-amino acid is α–carbon substituted with benzyl, substituted benzyl, indole, methylnaphthyl, cyclohexyl, methylcyclohexyl, indanyl, (C1-C5) alkyloxyaryl, aminoalkyl (C1-C8), guanidino alkyl (C1-C8), alkylcarboxylic, hydroxyalkyl (C1-C5), alkylthioalkyl, (C1-C3) alkylbenzamidio or hydrogen.

23. The method according to claim 20 wherein said aldehyde is of the formula R1C(O)H, wherein R1 is cyclohexyl, methylcyclohexyl, benzyl, substituted benzyl, phenethyl, biphenyl, benzoyl, hydrogen, methylnapthyl, indanyl, guanidinoalkyl (C2-C8), aminoalkyl (C2-C8), arylalkylcarbamate with alkyl chains of C2 to C8, alkanoyl (C1-C12) or alkyl (C1-C12).

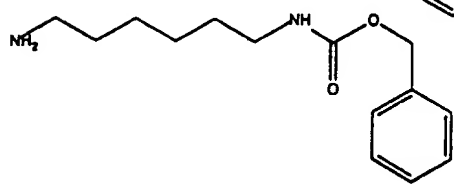
24. The method according to claim 20 wherein said primary amine is of the formula H2NR3 where R3 is selected from cyclohexyl, methylcyclohexyl, benzyl, substituted benzyl, phenethyl, biphenyl, benzoyl, hydrogen, methylnapthyl, indanyl, guanidinoalkyl (C2-C8), aminoalkyl (C2-C8), arylalkylcarbamates with alkyl chains of C2 to C8, alkanoyl (C1-C12), alkyl (C1-C12), (C2-C12)-alkylguanidinoalkyl (-C2-C8), benzamidino, (C1-C6) alkylbenzamidino, (C1-C6) alkylquinuclidine, (C1-C6) alkylpiperazine, C1 to C6 alkyl N-substituted piperazine, (C1-C6) alkylamino piperidine, (C1-C6) alkylamino N-substituted piperidine, (C1-C6) alkylcarboxyamidobenzamidino, (C1-C6) alkylcarboxamidoquinuclidine, (C1-C6) alkylcarboxamido piperazine, C1 to C6 alkylcarboxyl N-substituted piperazine, (C1-C6) alkylcarboxamidino piperidine and (C1-C6) alkylcarboxamidino N-substituted piperidine.

25. The method according to claim 20, wherein
W is 10;
X is 10; and
Z is 12.

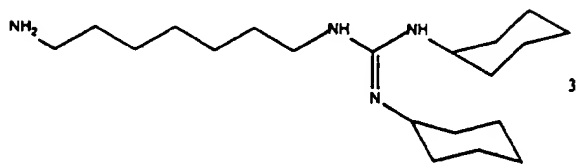
26. The method according to claim 25, wherein said α-amino acids are L- and D-Phenylanine, D- and L-Tryptophan, D- and L-Cyclohexylalanine, D- and L- ß-2-Naphthylalanine, and D- and L-Indanylglycine; said aldehydes and ketones are cyclohexanone, cyclohexanone carboxaldehyde, benzaldehyde, phenylacetalaldehyde, 4'-biphenylcarboxaldehyde, 2-Napthaldehyde, 2-indanone-, 2-(N-Cbz)-ethanal, 6-(N-Cbz)-hexanal and 12-(N-Cbz)-dodecanal; and said primary amines are



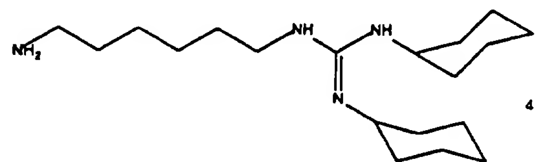
1



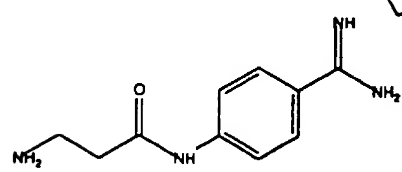
2



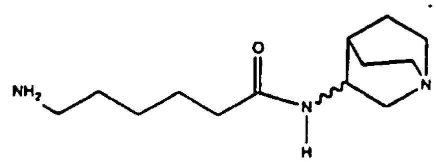
3



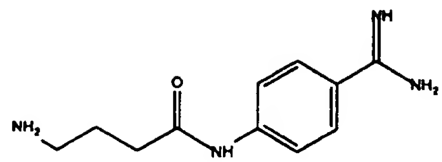
4



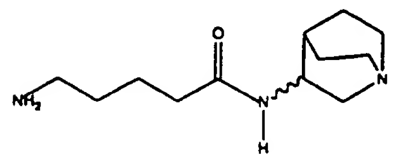
5



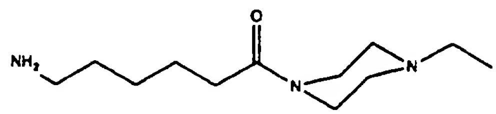
6



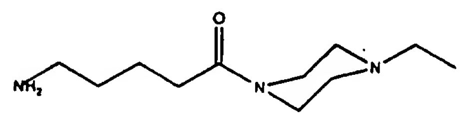
7



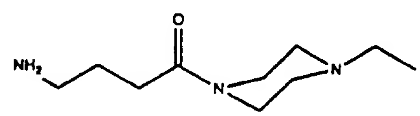
8



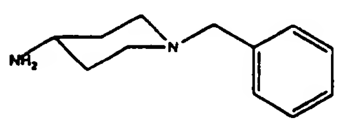
9



10



11

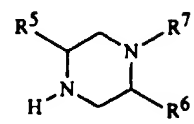


12

27. The N-substituted 1,4-Piperazines made according to claim 21.

28. The N-substituted 1,4-Piperazinediones made according to claim 19.

29. A compound of the formula:



wherein R^5 is benzyl or 2-indanyl; R^6 is $-(CH_2)_4NHC(=NH)NH_2$; and R^7 is 2-indanyl, $-CH_2C_6H_{11}$, benzyl, or $-CH_2CH_2Ph$.

30. The compound of claim 29 wherein R^5 is benzyl.

31. The compound of claim 30 wherein R^5 is R-benzyl.

32. The compound of claim 31 wherein R^6 is S $-(CH_2)_4NHC(=NH)NH_2$; and R^7 is 2-indanyl or $-CH_2C_6H_{11}$.

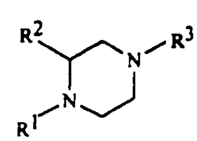
33. The compound of claim 31 wherein R^6 is R $-(CH_2)_4NHC(=NH)NH_2$; and R^7 is $-CH_2C_6H_{11}$ or $-CH_2CH_2Ph$.

34. The compound of claim 30 wherein R^5 is S-benzyl.

35. The compound of claim 34 wherein R^6 is S $-(CH_2)_4NHC(=NH)NH_2$; and R^7 is $-CH_2C_6H_{11}$, benzyl or $-CH_2CH_2Ph$.

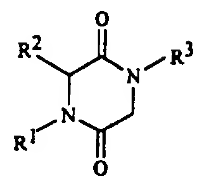
36. The compound of claim 29 wherein R^5 is 2-indanyl; and R^7 is $-CH_2C_6H_{11}$.

37. A compound of the formula:

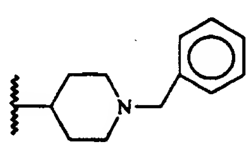


wherein R^1 is benzyl; R^2 is $-CH_2C_6H_{11}$; and R^3 is $-(CH_2)_7NHC(=NH)NH_2$ or $-(CH_2)_6NHC(=NH)NH_2$.

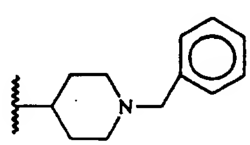
38. A compound of the formula:



wherein R^1 is $-CH_2C_6H_{11}$, $-CH_2$-2-Nph, or 2-indanyl; R^2 is $-CH_2CH_2Ph$, $-CH_2C_6H_{11}$, $-CH_2$-2-Nph or 4'-biphenyl; and R^3 is $-(CH_2)_7NHC(=NH)NH_2$, $-(CH_2)_6NHC(=NH)NH_2$ or



39. A compound of claim 38 wherein R^3 is



40. A compound of claim 39 wherein R^2 is $-CH_2CH_2Ph$.

41. A compound of claim 40 wherein R^1 is S $-CH_2C_6H_{11}$.

42. A compound of claim 40 wherein R^1 is R or S $-CH_2$-2-Nph.

43. A compound f claim 39 wherein R^1 is 2-indanyl and R^2 is $-CH_2C_6H_{11}$.

44. A compound of claim 38 wherein R^1 is 2-indanyl and R^2 is CH_2-2-Nph.

45. A compound of claim 44 wherein R^1 is S-2-indanyl and R_3 is $-(CH_2)_6NHC(=NH)NH_2$.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/14569

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D241/08 C07D295/02 A61K31/495

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB,A,2 223 227 (SOCIETE D'EXPANSION SCIENTIFIQUE EXPANSIA) 4 April 1990 see page 15 - page 17; claims ---	1
A	WO,A,95 04277 (SPHINX PHARMACEUTICALS CORPORATION) 9 February 1995 see page 53 - page 71; claims ---	1
A	BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 5, no. 1, 1995, pages 47-50, XP000613027 DAVID W. GORDON ET EL: cited in the application see the whole document ---	1-28
P,A	WO,A,96 00391 (AFFYMAX TECHNOLOGIES NV) 4 January 1996 see the whole document -----	1-28

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
16 January 1997	22.01.1997

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Luyten, H

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/14569

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
GB-A-2223227	04-04-90	AT-B-	400439	27-12-95
		AT-A-	219989	15-05-95
		AU-B-	622705	16-04-92
		AU-A-	4161489	29-03-90
		BE-A-	1002237	30-10-90
		CA-A-	1333441	06-12-94
		CH-A-	679672	31-03-92
		DE-A-	3931731	29-03-90
		FR-A-	2636951	30-03-90
		GR-B-	1000564	26-08-92
		HK-A-	47792	10-07-92
		IE-B-	62008	14-12-94
		JP-A-	2129200	17-05-90
		JP-B-	7068265	26-07-95
		LU-A-	87592	08-01-90
		NL-A-	8902361	17-04-90
		NO-B-	175593	25-07-94
		OA-A-	9243	30-06-92
		PT-B-	91784	31-01-96
		SE-A-	8903121	25-03-90
		US-A-	5175254	29-12-92
WO-A-9504277	09-02-95	AU-A-	7329394	28-02-95
		CA-A-	2168886	09-02-95
		EP-A-	0712493	22-05-96
		ZA-A-	9405731	07-03-95
WO-A-9600391	04-01-96	AU-A-	2871195	19-01-96
		AU-A-	2946895	15-01-96